THE BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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AUGUST TO DECEMBER, 1951

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Volume 101 Number 1

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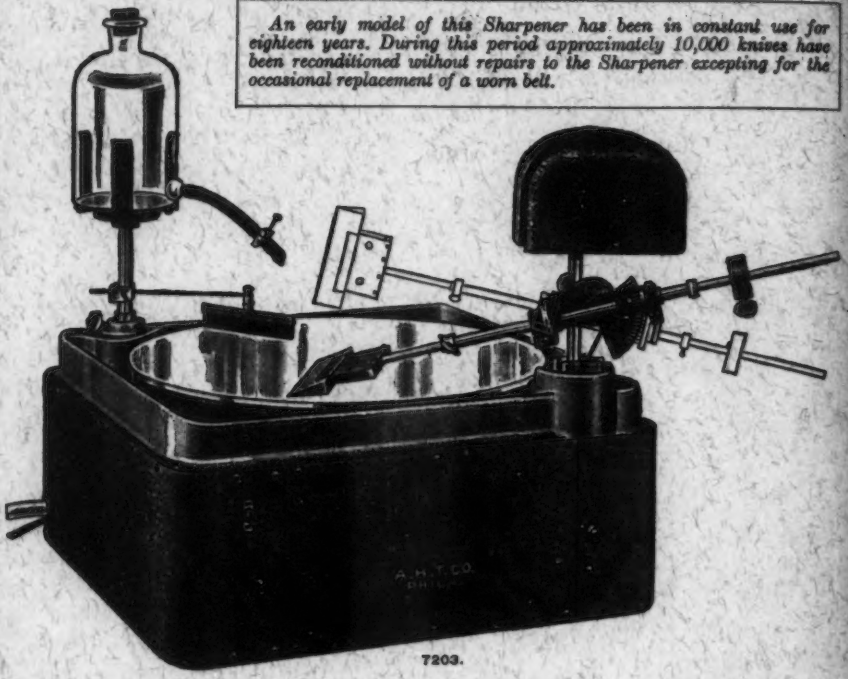
IMPROVED MODEL, FANZ AUTOMATIC

MICROTOME KNIFE SHARPENER

An early model of this Sharpener has been in constant use for eighteen years. During this period approximately 10,000 knives have been reconditioned without repairs to the Sharpener excepting for the occasional replacement of a worn belt.



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THE BIOLOGICAL BULLETIN

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THE MARINE BIOLOGICAL LABORATORY

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:00 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 9:00 A.M. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years. After having served two terms of four years each, Trustees are ineligible for re-election until a year has elapsed. In addition, there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk;

(C) Trustees Emeriti, who shall be elected from *present* or *former* Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. Any person interested in the Laboratory may be elected by the Trustees to a group to be known as Associates of the Marine Biological Laboratory.

XI. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XII. The account of the Treasurer shall be audited annually by a certified public accountant.

XIII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

The financial situation of the Laboratory at the end of 1950 was about the same as it was in December, 1949. Total cash receipts for the year amounted to $327,185.65 and expenditures, including $17,514.05 for capital assets, were $324,383.96. These totals were $19,550.51 and $14,283.03, respectively, in excess of the totals for 1949. The increases in charges for tuition, laboratory space and dormitory and Mess accommodations were just about sufficient to enable the Laboratory to meet increasing costs including a few very much needed salary and wage adjustments, but the margin is much too slim. Additional income for current operating expenses is still of the utmost urgency.

The statement of actual cash receipts and disbursements which has been given you in recent years is believed to be helpful in giving the overall picture, but in the case of some of the departments listed it may be misleading because of the fact that it does not include shares of the overhead cash expenses grouped under administration, buildings and grounds, etc. For instance, if overhead expense and depreciation are included, the Mess had a deficit of $2,145.52 and the dormitories a deficit of $1,258.68. Conversely, in the case of the Supply Department, when the cost of specimens furnished to Research and Instruction (estimated at $16,368.20) and other bookkeeping items are taken into account, there is a net income of $1,379.84 instead of the loss of $9,462.61 on a departmental cash basis.

Income from the Endowment Funds of the Laboratory increased $6,084.44 over last year to $46,431.59, due to larger common stock dividends. The average yield on securities was 4.8% on book value and 4.3% on market value. The investments and percentages in the Endowment Funds as of Jan. 1, 1951, were:

	Book Value	%	Market Value	%
Bonds				
U. S. Government	$367,997.66	38.31%	$ 361,447.87	33.90%
Railroad	54,613.02	5.65	59,662.50	5.60
Public Utility	90,192.50	9.34	89,706.25	8.41
Industrial	34,800.00	3.60	34,283.75	3.21
Total Bonds	$549,603.18	56.90	$ 545,100.37	51.12
Preferred Stocks	125,828.41	13.03	123,877.25	11.62
Common Stocks	290,371.21	30.07	397,386.25	37.26
Total Securities	$965,802.80	100.00%	$1,066,363.87	100.00%
Principal Cash	2,419.51		2,419.51	
Totals	$968,222.31		$1,068,783.38	

The Balance Sheet, Statement of Current Surplus, and Summary of Cash Transactions, as given in the report of the auditors, Seamans, Stetson and Tuttle of Boston, follow:

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DEC. 31, 1950

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee		$ 968,222.31	
Securities and Cash in Minor Funds		18,998.47	$ 987,220.78
Plant Assets:			
Land	$ 113,626.38		
Buildings	1,490,666.08		
Equipment	257,161.69		
Library	403,433.74		
	$2,264,887.89		
Less Reserve for Depreciation	786,616.68	$1,478,269.21	
Book Fund Cash		2,939.75	$1,481,208.96
Current Assets:			
Cash		$ 22,924.33	
Mortgage Note Receivable		2,350.00	
Accounts Receivable		19,874.97	
Inventories:			
Supply Department	$ 53,670.83		
"Biological Bulletin"	16,191.21	69,862.04	
Investments:			
Devil's Lane Property	35,648.04		
Stock in General Biological Supply House	12,700.00		
Other Investment Securities	46,109.29		
Retirement Fund	20,244.36	114,701.69	
Prepaid Insurance		7,056.77	
Items in Suspense (Debits)		1,161.41	$ 237,931.21
			$2,706,360.95

Liabilities

Endowment Funds:			
Endowment Funds	$ 966,445.89		
Reserve for Amortization	1,776.42	$ 968,222.31	
Minor Funds		18,998.47	$ 987,220.78
Plant Funds:			
Mortgage Notes Payable		$ 11,000.00	
Donations and Gifts	$1,331,699.04		
Other Investments in Plant from Gifts and Current Funds	138,509.92	1,470,208.96	$1,481,208.96
Current Liabilities and Surplus:			
Accounts Payable		$ 5,361.61	
Items in Suspense (Credits)		$ 1,055.00	
Current Surplus		231,514.60	$ 237,931.21
			$2,706,360.95

Current Surplus Account

Balance, January 1, 1950		$ 215,443.60
Add:		
Excess of Income over Expense for Year	$ 4,685.77	
Reserve for Depreciation, 1950, Charged to Plant Funds	29,160.29	33,846.06
		$ 249,289.66
Deduct:		
Payments from Current Funds during year for Plant Assets:		
Buildings	$ 779.45	
Equipment	5,773.72	
Library	10,221.89	
	$ 16,775.06	
Payment on Principal of Mortgage Note given in Partial Payment for "Dolphin"	1,000.00	17,775.06
Balance December 31, 1950		$ 231,514.60

Summary of Cash Transactions for Year ended December 31, 1950

		Expenditures		
Current Cash	Receipts	Current	Additions to Capital Assets	Total Expenditures
Income from Endowment Funds	$ 46,431.59			
Income from Other Investments	22,502.00			
Real Estate Rentals	6,864.64	$ 1,781.74		$ 1,781.74
Donations	24,532.51			
Membership Dues	2,460.00			
Instruction	16,811.15	10,073.86		10,073.86
Research (including Apparatus and Chemical Departments)	30,738.61	26,949.50	$ 2,929.06	29,878.56
Supply Department	74,079.32	82,896.48	645.45	83,541.93
Mess	48,513.37	47,123.02	964.50	48,087.52
Dormitories	22,824.85	10,541.46	467.96	11,009.42
"Biological Bulletin"	7,551.71	14,253.77		14,253.77
Library	4,974.91	8,591.71	9,874.48	18,466.19
Frank R. Lillie Memorial Fund—Arbacia Survey	5,129.49	4,235.87		4,235.87
Office of Naval Research	3,522.50	3,440.16		3,440.16
Miscellaneous	4,576.07	755.73		755.73
Book Purchase Account, Woods Hole Oceanographic Institution	800.00	838.79		838.79
Real Estate Sales (on account)	4,872.93			
Administration		24,718.91		24,718.91
Buildings and Grounds		51,010.98	1,632.60	52,643.58
Interest, Insurance and other Property Maintenance		10,769.50		10,769.50
Payments to Retirement Fund		8,888.43		8,888.43
Payment on Mortgage			1,000.00	1,000.00
	$327,185.65	$306,869.91	$17,514.05	$324,383.96

Cash Balance, January 1, 1950:		
Central Hanover Bank and Trust Co...	$ 8,159.11	
Falmouth National Bank.....	2,131.69	$ 10,290.80
Receipts.....		327,185.65
		$337,476.45
Payments		324,383.96
Cash Balance, December 31, 1950		
Central Hanover Bank and Trust Co....	$ 11,003.27	
Falmouth National Bank.....	2,089.22	$13,092.49

Other Cash Accounts	Balance Jan. 1, 1950	Received	Paid	Balance Dec. 31, 1950
Allen R. Memhard Fund Cash.....	$ 58.05	$ 25.00		$ 83.05
Rev. Arsenious Boyer Burse Cash.....	561.33	125.00	$ 504.46	182.07
Lucretia Crocker Fund Cash.....	1,747.88	440.43	1,513.39	674.92
Bathing Beach Fund Cash.....	1,000.00			1,000.00
Dr. Frank R. Lillie Memorial Fund Cash.	4,952.47	18,598.15	15,218.78	8,331.84
Old Main Building Fund Cash (Rockefeller).....	30,720.64		30,720.64	
Retirement Fund Cash.....	4,555.05	11,082.29	14,637.99	999.35
Bio Club Scholarship Fund Cash.....	130.03	62.50	120.00	72.53
Book Fund Cash.....	838.93	5,056.25	2,601.70	3,293.48
Lalor Foundation Fellowships Cash.....	2,118.21	5,000.00	5,057.28	2,060.93

Respectfully submitted,
DONALD M. BRODIE,
Treasurer

V. REPORT OF THE LIBRARIAN

The sum of $12,544 was appropriated to the Library for the year 1950, plus $3000 from the Woods Hole Oceanographic Institution to be applied to staff salaries. This amount was expended as follows: serials, $4427.38; books, $565.17; binding, $2100.87; supplies and sundries, $843.37; freight and express, $41.61; insurance, $55.00; and salaries, $7768.89. The total expenditures amounted to $15,802.29.

In addition to the above, $800 (plus a balance of $63.89 from 1949) was received from the Woods Hole Oceanographic Institution for acquisitions covering the field of oceanography. Of this amount, $804.79 was spent, leaving a balance of $59.10 on Dec. 31, 1950.

From the Carnegie Corporation of New York Fund, 24 completed back sets and 37 partially completed back sets were purchased, at a cost of $3078.49. The balance of this fund will be used during the coming year and a final summary of acquisitions realized from the $25,000 will be given in the 1951 report.

During the year, 1478 (66 new) current journals were received. Of these, 480 (17 new) were Marine Biological Laboratory subscriptions, 587 (24 new) were exchanges and 158 (13 new) were gifts; 69 (3 new) were Woods Hole Oceanographic Institution subscriptions; 161 (3 new) were exchange and 23 (6 new) were gifts.

The Marine Biological Laboratory purchased 66 books, received 5 complimentary copies from authors, 85 titles from publishing firms and 21 miscellaneous donations. The Woods Hole Oceanographic Institution purchased 6 titles, making a total of 183 books added to the shelves.

There were 27 back sets completed: 26 by purchase (2, Woods Hole Oceanographic Institution), and 1 by exchange; 51 were partially completed: 42 by purchase (5, Woods Hole Oceanographic Institution), 1 by exchange and 8 by gift.

The reprint additions to the Library numbered 5146. Of these, 2009 were of current issue and 3137 of earlier dates. Acknowledgment is made to Dr. Dorothy Wrinch and to Dr. A. M. Pappenheimer for gifts of reprints totalling 4780. Of these, 1299 were not already contained in the Library's collection.

Fifty volumes were borrowed on inter-library loan and 125 were sent to other institutions. Eighty-four microfilm orders were filled, amounting to $190.53.

The amount of $172.49 was realized from the sales room, and from this sum, new lights for the cataloguing room have been purchased.

There were two changes made in the staff during the year—Miss Benevides and Miss Hoffer joined the personnel, replacing Mrs. McInnes and Miss Goodfellow.

At the end of the year 1950, the Library contained 59,980 bound volumes and 174,081 reprints.

For the ever-willing help received from the Library Committee during 1950, the Librarian wishes to express her appreciation.

Respectfully submitted,
DEBORAH LAWRENCE HARLOW,
Librarian

VI. REPORT OF THE DIRECTOR

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY:

Gentlemen:

The sixty-third year of the Marine Biological Laboratory opened with a new administrative leadership and organizational set-up. The retirement of Dr. Charles Packard and of Mr. F. M. MacNaught, effective at the close of the preceding year, created serious gaps in the direction and management of the Laboratory. Fortunately we had available for the position of General Manager, Mr. Homer P. Smith who had had the valuable experience of serving as Assistant Business Manager with Mr. MacNaught.

Mr. Smith took over his duties as General Manager with energy and imagination. With the excellent cooperation of the Maintenance staff, he has continued the rehabilitation of the Laboratory plant initiated by Dr. Packard at the close of the war. Mr. Smith with the assistance of Mr. R. W. Kahler, has developed a long-range plan which, if it can be carried out in the present emergency and in the future, will result in the maintenance of our plant in first-rate condition.

The reorganization of the Laboratory administrative set-up, developed by a committee under the chairmanship of Dr. Eric G. Ball, has worked most satisfactorily. The lines of authority and responsibility are well defined, resulting in excellent cooperation and performance by the members of the permanent staff.

Mess Hall Management

During the winter Mr. Smith explored the possibilities of employing a new steward for the Mess Hall. Miss Belle Downing completed this season fifty years of summer service in the Mess Hall, the past several years in direct charge. These duties, combined with the supervision of the dormitory maid service, were too heavy for one person and Mr. Robert T. Martin, Manager of the dining hall at Harvard University's Adams House, took over as steward. He proved a most fortunate choice as Mr. Martin, assisted by Mr. John De Petris, has done an outstanding job. Increased cost of food and help necessitated an increase in Mess rates which was more than compensated by the improvement in the food and service.

Contributions and Gifts

Contributions to the Frank R. Lillie Memorial Fund during the year totaled $25,860.12. The Laboratory is much indebted to the contributors which included the Eli Lilly Company, $15,000; Dr. G. H. A. Clowes, $5,000; the Kettering Foundation, $2500; and the General Biological Supply House, $1000. A number of the Corporation members also contributed and it is hoped that other members will also give to the fund.

Contributions from the Associates totaled $1465, and, in addition, there was an anonymous contribution from one of the associates who donated $1000 to the Laboratory to be used as was deemed most advisable. With the Associates' contribution, the Laboratory has purchased a steam generator which has been urgently needed by the Laboratory for the past several years.

Dr. William Curtis, as has been his custom in the past, made a special donation to the Laboratory: this year, a handsome compass and an automatic heater for the *Dolphin*.

Grants

The American Philosophical Society has instituted a program through which they support three research rooms annually for the next five years. This grant amounts to $1800 each year and covers the total maintenance costs of these rooms. Already this has proved a real asset, providing space for visiting foreign investigators who frequently do not have funds available to cover the Laboratory charges.

The American Cancer Society continued its support of the "hot lab" by a grant which will provide experienced technical assistance so necessary in the handling of radioactive materials.

The Laboratory continues to receive support from the Rockefeller Foundation from a grant which has three more years to run. These funds supplement the regular Laboratory income for general operations and are very important in helping the Laboratory operate with reasonable effectiveness. This past summer we enjoyed a visit from President Chester I. Barnard of the Rockefeller Foundation and Dr. Warren Weaver, Director of the Natural Science Division. They spent two days surveying the research and teaching activities and facilities of the Laboratory. They were impressed by the need of the Laboratory for certain new items of equipment and apparatus.

Lalor Fellowship Program

Dr. C. Lalor Burdick visited the Laboratory in August, talked with the Lalor Fellows and met with the Fellowship Committee. Dr. Burdick encouraged the Committee to apply for a renewal of the fellowship grant and this was done. Notice has been received from the Foundation that the fellowship grant has been renewed for five years and increased to $6000 annually. The outstanding success of this program should encourage the Laboratory to seek additional funds for fellowships in fields not covered by the Lalor Grant.

Office of Naval Research Contract

A contract with the O. N. R. provided funds in the amount of $5000 for the collection, transportation, and distribution of squid for research purposes. A truck was especially equipped with a cooled, aerated, salt water tank. With this equipment, it has been possible to transport squid from the north side of the Cape to Woods Hole, the animals arriving at the Laboratory in excellent condition. The funds also provided a new engine for the *Tern*, which brings in squid from the Martha's Vineyard Sound fish traps.

Arbacia Program

There was a serious shortage of Arbacia for research purposes during the 1949 season. With funds from the Frank R. Lillie Memorial Fund, an Arbacia scouting project was developed in 1950, to locate new collecting beds. Dr. John S. Rankin spent the major part of the summer dredging likely areas and located a long, narrow bed off the northwest shore of Martha's Vineyard between Gay Head and Menimsha Bight. It is hoped that in the near future an ecological program may be developed for the investigation of Arbacia. Through the cooperative use of the Arbacia collected from the old beds, most investigators obtained adequate materials. Some investigators substituted for their research other forms which, in some cases, were more satisfactory than Arbacia.

Ecology Program

Early in the year a committee was set up under the Chairmanship of Dr. W. C. Allee to develop an ecological program. Such a program appeared desirable, particularly in view of our difficulty in obtaining some forms in adequate numbers for research and, still more, to broaden the base of operation of the Laboratory. As a first step the Committee recommended the establishment of a course in ecology at the graduate level for a limited number of students. This was approved by the Board of Trustees, provided funds could be obtained to support the course. The problem was discussed with Mr. Fairfield Osborn, President of the New York Zoological Society. This Society very generously has made a grant of $4000 a year for three years to assist us in getting this program under way.

Oceanographic Institution Expansion

There is urgent need for expansion of the Oceanographic Institution plant to take care of Navy contract research resulting from the present emergency. The most favorable site for this expansion is on the Bar Neck Property adjacent to the Oceanographic Institution. This property is owned by the Marine Biological

Laboratory. A majority of the M. B. L. Trustees oppose the sale of this unique piece of property, although there was considerable support for leasing the property. It should be possible to solve this problem to the mutual satisfaction of both institutions, permitting effective expansion of the Oceanographic Institution but, at the same time, protecting the interests of the Marine Biological Laboratory.

Biological Bulletin

At the termination of this year, Dr. Donald P. Costello became editor of the "Biological Bulletin," succeeding Dr. H. Burr Steinbach.

Housing

Housing continues to be a vexing problem though it has been eased somewhat by the building of several houses in the Devil's Lane Tract during the past two years. The roads in this tract have been accepted by the town and power lines and water mains have been installed.

There are appended as parts of this report:

1. Memorial
2. The Staff
3. Investigators and Students
4. The Lalor Fellows
5. Tabular View of Attendance
6. Subscribing and Cooperating Institutions
7. Evening Lectures
8. Shorter Scientific Papers Presented at the Seminar
9. Members of the Corporation

Respectfully submitted,
PHILIP B. ARMSTRONG,
Director

1. MEMORIAL

Leonor Michaelis

By

E. S. G. BARRON

The Marine Biological Laboratory mourns the death of Professor Michaelis. The loss is more deeply felt, as his death came just two months after the Department of Physiology had decided to celebrate his 75th birthday by dedicating to him the Physiology Course of 1950. He was very touched by this tribute and had accepted our invitation to spend the summer with us. Students and staff expected to profit from his genius and his wisdom. A member of the Corporation since 1928, Professor Michaelis was an instructor of the Physiology Course for ten years.

Born in Berlin, Germany, on January 16, 1875, Leonor Michaelis graduated from the Medical School in Berlin in 1897. That his life was to be devoted to the pursuit of knowledge was demonstrated early. While still a student he published a paper on the histology of milk secretion which was rewarded with a prize from the Medical School,

the only recognition that was to be awarded to him in time. In Professor Hertwig's laboratory young Michaelis worked on embryological problems; his paper on the cytology of the fertilization of the ovum of the amphibian *Triton taeniatus*, as well as his doctor's thesis on the determination of the direction of the first cleavage in the frog's egg, reveals rare insight and clarity of conception.

It was natural that Michaelis' early scientific steps were directed toward microscopic anatomy, since medical thinking at that time was strongly influenced by the rapid advances made with the aid of the microscope. Perhaps he would have directed himself toward pathology if, "by some strange coincidence" as he says it, he had not met Paul Ehrlich, who at that time was looking for a young histologist. Ehrlich offered him a one-year position as his private assistant in the State Institute for Serological Testing and Research. It was here that Michaelis studied the chemistry of staining, and it was here that the vital staining of mitochondria with Janus Green originated. There is no doubt that this one year at Ehrlich's laboratory exerted a decisive influence on the direction of his investigations. The whole field of physico-chemical sciences as applied to biology and medicine was here opened to him. With his powerful and quick intellect he visualized the unlimited applications of physics and chemistry to biological phenomena. The science of Biochemistry at the start of the century, when Michaelis entered Ehrlich's laboratory, was still in its infancy, mostly sheltered in physiological and pathological laboratories; and the *Journal of Biological Chemistry, Biochemical Journal* and *Biochemische Zeitschrift* did not appear until six years later. Here, in front of him, was a field of vast possibilities, and he with no financial facilities, and no formal, classical training to enter it. Indeed Ehrlich himself had advised him to enter clinical medicine, which he had to do, becoming an assistant to Professor Litten in a department of one of the municipal hospitals in Berlin from 1900 to 1904. A herculean task was this, taking care of the sick and at the same time preparing himself with the solid foundations required for fundamental research in quantitative biology. With no teachers to guide him, armed only with his genius and his tenacious perseverance, he studied chemistry, as he once told me, in a dictionary, and mastered the branches of mathematics necessary to become familiar with the solution of physicochemical problems. And while thus schooling himself for the task ahead, he still found time to continue his investigations in histology and staining.

In 1903 he was made a *Privadozent* at the University of Berlin with a thesis on immunological protein precipitins. By combining enzyme techniques he observed that short digestion of serum albumin with pepsin changed profoundly the immunological properties. He was also the first to show that aqueous extracts of normal livers could be used for the Wassermann test, and that the complement fixation test in the Wassermann reaction could be replaced by a direct precipitation test by using extracts of syphilitic foetus of high potency.

In 1904, a paper about some iron-containing inclusions in tumors of the urinary bladder aroused the interest of Professor v. Leyden who appointed him research assistant for the newly created Institute for Cancer Research at the University Clinic. He worked here on experimental cancer, and once was sent to Jena to bring the ultramicroscope newly built by Siedenhopf and Zsigmondy at the Zeiss optical works. With this instrument v. Leyden had great hopes of finding the cause of cancer; Michaelis had great hopes of utilizing the instrument for studies of colloidal chemistry (which he indeed succeeded in doing).

In 1905, Michaelis received the title of Professor. What could a Professor do with no salary, no laboratory, no funds to support his work? Fully realizing the hopelessness of this situation, he accepted the position of bacteriologist at the municipal hospital "am Urban" in Berlin, which brought him in contact with Peter Rona, the chemist of the hospital. The close friendship and association with Rona lasted throughout his seventeen-

year stay at the hospital. It was here that the full powers of Michaelis came to glorious eclosion. In his humble research room, with barely space enough—as Albert Szent-Györgyi has said to me—for one man to walk through, and with scarce equipment, Michaelis accomplished, either alone or in collaboration with his friend Peter Rona and a number of young scientists from abroad and from Germany, the series of remarkable investigations on the ionic environment, on weak electrolytes, on adsorption, on proteins, on enzymes—chapters which are now the secure foundation of quantitative biology.

Simultaneously with Sørensen, Michaelis foresaw the influence of the hydrogen ion concentration on biological phenomena and proceeded to develop the theory and practice of hydrogen ions. The hydrogen electrode he built for exact measurements. The theory of buffers, or, as he called them, "hydrogen ion regulators," was lucidly presented. The theories of the dissociation of amphotheric electrolytes, and of the iso-electric points of amino acids and proteins were developed quantitatively, after Hardy had introduced in a qualitative way the concept of the isoelectric point. He developed the method of electrophoresis in such a way as to keep the pH constant during the flow of current. The isoelectric points of casein, hemoglobin, serum albumin, serum globulin, native and denatured globin, gelatine, and edestin were thus determined. He was the first to show that the charge of a colloid, enzyme, or even of whole bacteria was not a property inherent in the substance alone but was dependent upon the pH and that there is an isoelectric point characteristic of the substance.

He was devoting his attention at the same time to the field of enzymes. Mindful of the influence of ionic environment, he studied the effect of the hydrogen ion concentration on enzyme activity, and although Sørensen published first similar studies, Michaelis proceeded to extend Sørensen's observations by showing that the dependence of enzyme activity on pH was of the same nature as the dependence of the dissociation of a weak acid on pH. By comparing the enzymatic activity at constant pH but varied concentrations of substrate he presented lucidly and quantitatively the concept of affinity constant. And thus the theory of enzyme-substrate combination, postulated by Henry in 1903, was established on a firm basis ten years later, in 1913. At a time when the protein nature of enzymes was as yet unknown, Michaelis guided by his genius foresaw it, and with daring and his elegant simplicity put forth the theory of enzyme-substrate complex formation. Now the Michaelis enzyme-substrate complex is one of the solid foundations of enzymology.

In a study of the effect of adsorption on enzyme activity he gave evidence of site specificity on the enzyme surface. And in his studies on the nature of enzyme inhibitors he distinguished the competitive inhibitors from those which influence the rate of splitting of the enzyme-substrate into the split products and free enzyme.

Studies on adsorption, on bacterial agglutination, on blood sugar, on buffers, and on pH indicators crowded those years of intense production.

The international reputation of Michaelis was widespread. In 1921 he received the title of *professor extraordinarius* of physical chemistry applied to medicine and biology at the University of Berlin. Again a professorship with no salary, no laboratory, no budget. Grim irony this "appointment" must have seemed to him! Hence, he accepted in 1921 the position of consultant in an industrial concern for scientific apparatus where he was provided with laboratory facilities. Less than one year later, however, he was invited to the chair of biochemistry at the re-organized medical school of Nagoya. Three pleasant and fruitful years were spent in Japan, where he initiated, in collaboration with Fujita, his studies on membrane permeability. Struck by the selective permeability of cell membranes, he strove to construct a model which would show similar properties, and thus he made his dried collodium membranes permeable only to cations. Here again he gave proof of his rare insight, for he terminated these studies upon recognizing that his models did not reproduce the properties of cellular membranes.

During his stay in Japan, Michaelis received an invitation from Jacques Loeb for a lecture tour in the United States, to be given in the summer of 1924. On the very day of his departure from Japan he received the tragic news of Jacques Loeb's sudden death. The lectures, however, had all been properly arranged, and on that occasion Michaelis was asked to come to Johns Hopkins as a resident lecturer for three years, beginning in the Spring of 1926 at the termination of his contract in Japan.

And thus, at Hopkins, started the third cycle of Michaelis' achievements. The field of oxidation- reduction processes and of heavy metal catalysis now received most of his attention. Here, as in the field of enzymes, his genius brought forth theories of fundamental importance for the understanding of the kinetics of biological oxidations. The simultaneous discovery with Elema, from Holland, of the two-step reduction of pyocyanine in acid solutions was the starting point of Michaelis' principle of monovalent oxidation-reductions and the intermediary formation of free radicals during the oxidation process of systems which perform the reaction by transfer of two electrons. The presence of such free radicals, existing in equilibrium with their "parent substances" even in aqueous solutions, was a daring concept which appeared absurd to conservative chemists. Like his theory of enzyme-substrate formation, it was more the foresight of a genius than a methodical and secure approach. The first papers on the theory of two-step oxidations were rejected by both the *Journal of Biological Chemistry* and the *Journal of the American Chemical Society*. So indignant was one referee of the latter journal that in horror he wrote, "In short, a principle of modern scientific philosophy is violated." The paper was published in *Biochemische Zeitschrift*. Michaelis felt deeply this attack. To answer the criticisms of lack of conclusive evidence and of too much reliance on potentiometric data, he diligently started the study of quantum mechanics and of magneto-chemistry, attacking them with the same enthusiasm as when thirty years previously he had prepared himself in organic chemistry and mathematics. He simplified the method of the magnetic balance for the measurement of the paramagnetism of these intermediate compounds and left no room for doubt about the existence of free radicals. Thus, the name of Michaelis was incorporated in every modern text book on organic chemistry.

Yet official recognition came always late. He became a member of the National Academy of Science five years after his retirement as active member of the Rockefeller Institute, where he spent the last years of his life—from the end of his lectureship at Johns Hopkins until his death in October of 1949.

With the death of Michaelis we lose a man of an intellectual stature that is more and more rare in this age of narrow specialization. He had the priceless gift of transforming the most abstruse and complex concepts into simple and clear ideas; the books he wrote were models of clarity and conciseness. His textbook on embryology reached nine editions. *Hydrogen Ion Concentrations, Manual of Physical Chemistry, Introduction to Mathematics for Biologists and Chemists* and *Oxidation-reduction Potentials* were all translated into various languages. His brilliant genius allowed him to perceive immediately the important points in any discussion, and his wide knowledge in the fields of biology, medicine, chemistry, physics, and mathematics, was always at the service of those who went to him for advice.

The humanistic type of Gymnasium where Michaelis received his secondary education broadened his intellectual training and interests. With no difficulties in Latin or Greek he tackled the Japanese language when in Japan and became quite a connoisseur of the old Oriental civilizations. Music attracted him greatly. In his first semester at the University he even took a course in counterpoint with Professor Bellerman. His improvisations at the piano were always a delight to his listeners.

The Marine Biological Laboratory mourns deeply the loss of a great man of science and a scholar of broad interests, as well as a friend of many years' standing.

2. The Staff, 1950

Philip B. Armstrong, Director, State University of New York, Medical Center at Syracuse.

Senior Staff of Investigation

E. G. Conklin, Professor of Zoology, *Emeritus,* Princeton University.
W. E. Garrey, Professor of Physiology, *Emeritus,* Vanderbilt University Medical School.
R. S. Lillie, Professor of General Physiology, *Emeritus,* The University of Chicago.
A. P. Mathews, Professor of Biochemistry, *Emeritus,* University of Cincinnati.
G. H. Parker, Professor of Zoology, *Emeritus,* Harvard University.

ZOOLOGY

I. Consultants

F. A. Brown, Jr., Professor of Zoology, Northwestern University.
Libbie H. Hyman, American Museum of Natural History.
A. C. Redfield, Woods Hole Oceanographic Institution.

II. Instructors

L. H. Kleinholz, Associate Professor of Biology, Reed College, in charge of course.
W. D. Burbanck, Professor of Biology, Drury College.
C. G. Goodchild, Professor of Biology, S. W. Missouri State College.
John H. Lochhead, Assistant Professor of Zoology, University of Vermont.
Madelene E. Pierce, Associate Professor of Zoology, Vassar College.
W. M. Reid, Professor of Biology, Monmouth College.
T. H. Waterman, Assistant Professor in Biology, Yale University.
Marion H. Pettibone, Arctic Research Laboratory, U. S. National Museum.

III. Laboratory Assistants

Robert S. Howard, Northwestern University.
Muriel Sandeen, Northwestern University.

EMBRYOLOGY

I. Instructors

Donald P. Costello, Kenan Professor of Zoology, University of North Carolina, in charge of course.
Arthur L. Colwin, Assistant Professor of Zoology, Queens College.
Charles B. Metz, Assistant Professor of Zoology, Yale University.
James A. Miller, Professor of Anatomy, Emory University.
S. Meryl Rose, Associate Professor of Zoology, University of Illinois.
Albert Tyler, Associate Professor of Embryology, California Institute of Technology.

II. Research Assistant

Margaret E. Davidson, McGill University.

III. Laboratory Assistants

J. Bruce Guyselman, Northwestern University.
Donald E. Kent, University of North Carolina.

PHYSIOLOGY

I. Consultants

Eric G. Ball, Professor of Biochemistry, Harvard University Medical School.
Merkel H. Jacobs, Professor of Physiology, University of Pennsylvania.
Otto Loewi, Professor of Pharmacology, New York University, School of Medicine.
Arthur K. Parpart, Professor of Biology, Princeton University.

II. Instructors

E. S. Guzman Barron, Associate Professor of Biochemistry, University of Chicago, in charge of course.
Daniel Mazia, Professor of Zoology, University of Missouri.
Hans Neurath, Professor of Biochemistry, Duke University Medical School.
John Runnström, Director of the Wenner Grens Institute, Stockholm.
H. Burr Steinbach, Professor of Zoology, University of Minnesota.
George Wald, Professor of Biology, Harvard University.

III. Laboratory Assistant

Patricia McIntyre, Johns Hopkins Medical School.

BOTANY

I. Consultants

Bostwick H. Ketchum, Woods Hole Oceanographic Institution.
Wm. Randolph Taylor, Professor of Botany, University of Michigan.

II. Instructors

Maxwell S. Doty, Assistant Professor of Biology, Northwestern University, in charge of course.
George J. Hollenberg, Professor of Biology, University of Redlands.
R. D. Wood, Assistant Professor of Botany, Rhode Island State College.

III. Laboratory Assistants

Ralph A. Lewin, Yale University.
Cecilia Rieger, Vassar College.

IV. Collector

Hannah T. Croasdale, Research Associate, Dartmouth College.

V. Lecturers

J. B. Lackey, Medical and Scientific Editor, The Blakiston Company.
Ruth Patrick, Curator of Limnology, Academy of Natural Sciences of Philadelphia.

EXPERIMENTAL RADIOLOGY

G. Failla, College of Physicians and Surgeons, Columbia University.
L. Robinson Hyde, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

Mrs. Deborah Lawrence Harlow, Librarian
Mary A. Rohan Loretta J. Benevides Joan M. Hoffer

APPARATUS DEPARTMENT

Robert Mills, Manager
James D. Graham Elizabeth J. Keeler
James D. Graham, Jr. Seaver R. Harlow

CHEMICAL DEPARTMENT

Gail M. Cavanaugh, Chemical Supplies Manager

SUPPLY DEPARTMENT

James McInnis, Manager
John S. Rankin, Naturalist
Ruth Crowell Janice McLaughlin
M. B. Gray G. Lehy James Whitcomb
A. M. Hilton Carl O. Schweidenback H. S. Wagstaff
W. E. Kahler R. O. Lehy Robert Perry

GENERAL OFFICE

Homer P. Smith, General Manager
Polly L. Crowell, Office Manager
Mrs. Lila S. Myers Miss Mary M. McLaughlin
Mrs. Norma Bonito

GENERAL MAINTENANCE

R. W. Kahler, Superintendent
Robert Adams James S. Thayer
Richard Alberts Albert Neal
Robert Gunning Alton J. Pierce
John H. Head T. E. Tawell
George A. Kahler

3. Investigators and Students

Independent Investigators, 1950

Abrams, Richard, Assistant Professor, Inst. Radiobiology and Biophysics, University of Chicago.
Allen, M. Jean, Assistant Professor of Zoology, University of New Hampshire.
Alscher, Ruth P., Assistant Professor, Manhattanville College of the Sacred Heart.
Armstrong, Philip B., Professor of Anatomy, State College of New York, Medical Center, Syracuse, N. Y.
Atwood, Kimball C., Visiting Professor of Zoology, Columbia University.
Baily, Norman A., Research Scientist, Columbia University.
Baker, Gladys E., Chairman, Plant Science Department, Vassar College.
Ball, Eric G., Professor of Biological Chemistry, Harvard Medical School.
Barnett, Robert C., Graduate Student in Physiology, University of Chicago.
Barron, E. S. Guzman, Professor of Biochemistry, University of Chicago.
Belford, Julius, Assistant Professor of Pharmacology, Brooklyn 2, New York.

BERGER, CHARLES A., Director, Biological Laboratory, Fordham University.
BERNSTEIN, MAURICE H., Graduate Assistant, Washington University.
BLISS, ALFRED F., Associate Professor of Physiology, Tufts College Medical School.
BLUM, HAROLD F., Physiologist, Princeton University.
BOETTIGER, EDWARD C., Assistant Professor of Zoology, University of Connecticut.
BONNER, JOHN TYLER, Assistant Professor, Princeton University.
VON BORSTEL, ROBERT C., Assistant Instructor, University of Pennsylvania.
BRIDGMAN, ANNA JOSEPHINE, Associate Professor of Biology, Agnes Scott College.
BROOKS, MATILDA M., Research Associate in Physiology, University of California.
BRONK, DETLEV, President, Johns Hopkins University.
BROWN, FRANK A., JR., Professor and Chairman Biological Sciences, Northwestern University.
BRUNST, V. V., Research Associate, University of Maryland Medical School.
BRUST, MANFRED, Research Assistant in Physiology, University of Chicago.
BUBIENIEC, ERNEST J., Graduate Student, University of Wisconsin.
BURBANCK, W. D., Chairman, Department of Biology, Drury College.
CAMBER, BERNARD, Fellow of the Rockefeller Institute for Medical Research.
CHAMBERS, EDWARD S., New York University, Washington Square College.
CHAMBERS, ROBERT, Professor *Emeritus*, New York University, Washington Square College.
CHARLES, DONALD R., Chairman, Division of Biological Sciences, University of Rochester.
CHASE, AURIN M., Associate Professor of Biology, Princeton University.
CHENEY, RALPH HOLT, Professor of Biology, Brooklyn College.
CLAFF, C. LLOYD, Research Associate in Surgery, Harvard Medical School.
CLARK, ARNOLD M., Assistant Professor of Biology, University of Delaware.
CLEMENT, A. C., Associate Professor of Biology, Emory University.
CLOWES, G. H. A., Research Director, *Emeritus*, Eli Lilly and Company.
COHEN, SEYMOUR STANLEY, Associate Professor of Physiological Chemistry, University of Pennsylvania.
COLWIN, ARTHUR L., Assistant Professor of Biology, Queens College.
COLWIN, LAURA N., Queens College.
CONKLIN, EDWIN G., Professor of Biology *Emeritus*, Princeton University.
COOPERSTEIN, SHERWIN J., Instructor of Anatomy, Western Reserve University.
COPELAND, D. EUGENE, Assistant Professor of Biology, Brown University.
CORNMAN, IVOR, Assistant Research Professor, George Washington University.
COSTELLO, DONALD PAUL, Kenan Professor of Zoology, University of North Carolina.
COYLE, ELIZABETH E., Associate Professor of Biology, College of Wooster.
CROWELL, SEARS, Assistant Professor of Zoology, Indiana University.
CURTIS, HOWARD J., Professor of Physiology, Vanderbilt University School of Medicine.
CURTIS, W. C., Professor of Zoology *Emeritus*, University of Missouri.
DEFALCO, RALPH, Associate Professor of Zoology, Rutgers University.
DOTY, MAXWELL S., Associate Professor of Biology, Northwestern University.
ELLIOTT, ALFRED M., Assistant Professor of Zoology, University of Michigan.
FAURÉ-FREMIET, EMMANUEL, Professor, Collège de France.
FINKELSTEIN, PAUL, AEC Postdoctoral Fellow, University of Chicago.
FOSTER, RICHARD W., Assistant Curator of Mollusks, Museum of Comparative Zoology, Harvard University.
GAFFRON, HANS, Associate Professor of Biochemistry, University of Chicago.
GARREY, WALTER EUGENE, Professor of Physiology *Emeritus*, Vanderbilt University School of Medicine.
GASVODA, BETTY, Junior Biochemist, Argonne National Laboratory.
GHOSH, BARENDRA N., Fellow of the Muscle Research, Woods Hole, Mass.
GILMAN, LAUREN C., Associate Professor of Zoology, University of Miami.
GLASER, OTTO, E. S. Harkness Professor *Emeritus*, Amherst College.
GOODCHILD, C. G., Professor of Zoology, Southwest Missouri State College.
GRAY, I. E., Professor of Zoology, Duke University.
GREEN, JAMES W., Assistant Professor of Physiology, Rutgers University.
GROSCH, DANIEL S., Assistant Professor of Zoology, North Carolina State College.
GRUNDFEST, HARRY, Associate Professor of Neurology, Columbia University.
HAJDU, STEPHEN, Associate Professor, Institute for Muscle Research.
HARDING, CLIFFORD V., Research Fellow, University of Pennsylvania.

HARVEY, ETHEL BROWNE, Independent Investigator of Biology, Princeton University.
HARVEY, E. NEWTON, Professor of Physiology, Princeton University.
HAYWOOD, CHARLOTTE, Professor of Physiology, Mount Holyoke College.
HEILBRUNN, L. V., Professor of Zoology, University of Pennsylvania.
HENLEY, CATHERINE, Research Associate, University of North Carolina.
HODES, ROBERT, Professor of Neurophysiology, Tulane University.
HOLTFRETER, JOHANNES, Professor of Zoology, University of Rochester.
INOUÉ, SHINYA, Graduate Student, Princeton University.
JACOBS, M. H., Professor of General Physiology, University of Pennsylvania.
JACOBS, WILLIAM P., Assistant Professor of Biology, Princeton University.
JENKINS, GEORGE B., Professor of Anatomy *Emeritus*, George Washington University.
KARUSH, FRED, Visiting Scholar, Columbia University.
KEEFFE, MARY M., Assistant Professor of Biology, College of St. Thomas.
KELLY, SALLY M., Assistant Professor of Plant Science, Vassar College.
KEMPTON, RUDOLF T., Professor of Zoology, Vassar College.
KING, JOHN W., Professor of Physiology, Morgan State College.
KISCH, BRUNO, Professor of Chemistry, Yeshiva University.
KLEINHOLZ, LEWIS H., Associate Professor of Biology, Reed College.
KLOTZ, IRVING M., Associate Professor of Chemistry and Biology, Northwestern University.
KRAHL, MAURICE E., Associate Professor of Biological Chemistry, Washington University, School of Medicine.
KREEZER, GEORGE L., Associate Professor, Washington University.
KUFFLER, STEPHEN W., Associate Professor, Wilmer Institute.
KUNTZ, ELOISE, Instructor, Vassar College.
LAJTHA, ABEL, Assistant Professor, Institute for Muscle Research.
LANE, CHARLES E., Research Associate, University of Miami.
LAZAROW, ARNOLD, Associate Professor of Anatomy, Western Reserve University.
LEVY, MILTON, Associate Professor of Chemistry, New York University College of Medicine.
LILLIE, RALPH S., Professor of Physiology *Emeritus*, University of Chicago.
LOCHHEAD, JOHN H., Associate Professor of Zoology, University of Vermont.
LOVELACE, ROBERTA, Adjunct Professor in Biology, University of South Carolina.
LUCKÉ, BALDUIN, Professor of Pathology, University of Pennsylvania.
MARSHAK, ALFRED, Adjunct Associate Professor, New York University College of Medicine.
MARSLAND, DOUGLAS, Professor of Biology, New York University, Washington Square College.
MARMONT, GEORGE, Assistant Professor of Physiology, University of Chicago.
MAZIA, DANIEL, Professor of Zoology, University of Missouri.
MEHLER, ALAN H., Research Associate, University of Chicago.
METZ, CHARLES B., Assistant Professor of Zoology, Yale University.
METZ, CHARLES W., Chairman, Department of Zoology, University of Pennsylvania.
MIHÁLYI, ELEMER, Research Assistant, Institute for Muscle Research.
MILLER, JAMES A., Professor of Anatomy, Emory University.
MILLOTT, NORMAN, Professor of Zoology, University College of West Indies.
MONROY, ALBERTO, Visiting Investigator, Zoological Station, Naples, Italy.
MOORE, GEORGE M., Chairman, Department of Zoology, University of New Hampshire.
NACHMANSOHN, DAVID, Assistant Professor of Neurology, Columbia University.
NELSON, LEONARD, Instructor in Zoology, University of Nebraska.
NEUBERG, CARL, Visiting Professor, Polytechnic Institute of Brooklyn.
NEURATH, HANS, Professor of Physical Biochemistry, Duke University.
NICOLL, PAUL ANDREW, Associate Professor of Physiology, Indiana University.
PALAY, SANFORD L., Instructor in Anatomy, Yale University, School of Medicine.
PARMENTER, C. L., Professor of Zoology, University of Pennsylvania.
PARPART, ARTHUR K., Chairman, Department of Biology, Princeton University.
PETTIBONE, MARIAN H., Researcher, Arctic Research Laboratory, U. S. National Museum, Washington, D. C.
PIERCE, MADELENE E., Associate Professor of Zoology, Vassar College.
PLOUGH, H. H., Professor of Biology, Amherst College.
PROSSER, C. LADD, Professor of Physiology, University of Illinois.
REDFIELD, ROBERT R., AEC Post-Doctorate Fellow, University of Chicago.
REID, W. MALCOLM, Head of Department of Biology, Monmouth College.

REISCHER, HELEN S., Research Associate, Haskins Laboratories.
RICCA, RENATO A., Clinical Assistant, Hartford Hospital.
RICH, ALEXANDER, Research Fellow in Chemistry, California Institute of Technology.
RIESER, PETER, Instructor in Physiology, Fordham University.
RONKIN, R. R., Assistant Professor of Physiology, University of Delaware.
ROSSI, HAROLD H., Assistant Professor of Radiology, Columbia University.
ROTH, JAY S., Assistant Professor of Biochemistry, Rutgers University.
ROTHENBERG, M. A., Research Associate, University of Chicago.
RUDENBERG, F. HERMANN, Graduate Student, University of Chicago.
RUNNSTRÖM, J., Director, Wenner-Grens Institut, Stockholm, Sweden.
RUTSTEIN, DAVID D., Professor of Preventive Medicine, Harvard Medical School.
SANDOW, ALEXANDER, Associate Professor of Biology, Washington Square College.
SARKAR, NIRMAL K., Research Assistant, Institute for Muscle Research.
SCHAEFFER, ASA A., Professor of Biology, Temple University.
SCHMITT, FRANCIS O., Head, Department of Biology, Massachusetts Institute of Technology.
SCHMITT, OTTO H., Professor of Zoology and Physics, University of Minnesota.
SCOTT, ALLAN C., Associate Professor of Biology, Union College.
SCOTT, SISTER FLORENCE MARIE, Professor of Biology, Seton Hill College.
SCOTT, GEORGE T., Associate Professor of Zoology, Oberlin College.
SEAMAN, GERALD R., Instructor in Biological Chemistry, School of Medicine, Creighton University.
SHANES, A. M., Associate Professor of Physiology and Biophysics, Georgetown University, School of Medicine.
SICHEL, F., Professor of Physiology and Biophysics, University of Vermont, College of Medicine.
SPEIDEL, CARL C., Professor of Anatomy, University of Virginia.
SPRATT, NELSON T., Associate Professor of Zoology, University of Minnesota.
STEINBACH, H. BURR, Professor of Zoology, University of Minnesota.
STERN, KURT G., Adjunct Professor of Biochemistry, Polytechnic Institute of Brooklyn.
STOKEY, ALMA G., Professor of Plant Science *Emeritus*, Mount Holyoke College.
STOUDT, HARRY N., Assistant Professor of Biology, Temple University.
STRAUS, WILLIAM L., Associate Professor of Anatomy, Johns Hopkins University School of Medicine.
STUNKARD, HORACE W., Professor of Biology, New York University.
STURTEVANT, A. H., Professor of Genetics, California Institute of Technology.
SZENT-GYÖRGYI, ALBERT, Institute for Advanced Studies, Princeton.
SZENT-GYÖRGYI, ANDREW, Institute for Muscle Research.
TAYLOR, WILLIAM RANDOLPH, Professor of Botany, University of Michigan.
TOBIAS, JULIAN M., Assistant Professor of Physiology, University of Chicago.
TRACY, HENRY C., Professor of Anatomy, University of Kansas.
TRINKAUS, J. P., Instructor in Zoology, Yale University.
TRUANT, ALDO P., Assistant Professor of Pharmacology, Tufts College Medical School.
TYLER, ALBERT, Associate Professor of Embryology, California Institute of Technology.
VARGA, LASZLO, Institute for Muscle Research.
VILLEE, CLAUDE A., JR., Assistant Professor of Biological Chemistry, Harvard Medical School.
VINCENT, WALTER S., JR., Atomic Energy Commission Fellow, University of Pennsylvania.
VISHNIAC, WOLF, Research Associate, New York University Bellevue Medical Center.
WARNER, ROBERT C., Assistant Professor of Chemistry, New York University College of Medicine.
WHITING, P. W., Professor of Zoology, University of Pennsylvania.
WICHTERMAN, RALPH, Associate Professor of Biology, Temple University.
WILBER, CHARLES G., Director, Biological Laboratories, St. Louis University.
WILDER, VIOLET M., Assistant Professor of Biochemistry, University of Nebraska.
WILSON, WALTER L., Instructor in Physiology and Biophysics, University of Vermont, College of Medicine.
WOOD, RICHARD, Assistant Professor of Botany, Rhode Island State College.
WOODWARD, ARTHUR A., JR., Assistant Professor of Zoology, Washington University.
WRINCH, DOROTHY, Lecturer in Physics, Smith College.
ZORZOLI, ANITA, Assistant Professor of Physiology, Washington University School of Dentistry.

Beginning Investigators, 1950

AARONSON, SHELDON, Research Associate, Haskins Laboratories.
ALLEN, ROBERT DAY, Assistant Instructor in Zoology, University of Pennsylvania.
BREEN, MOIRA, Assistant in Physiology, Vassar College.
BRUMMETT, ANNA RUTH, Fellow in Biology, Bryn Mawr College.
CANTOR, NANCY JOAN, University of Vermont, College of Medicine.
CLARK, MARGUERITE R., Graduate Fellow, St. Louis University.
COLÓN, DORIS E., Graduate Student, University of Pennsylvania.
EDGECOMBE, CAROLYN A., Student, University of Connecticut.
ESSNER, EDWARD S., Assistant Instructor, University of Pennsylvania.
GAGNON, ANDRÉ, Assistant Instructor, University of Pennsylvania.
GOLDSTEIN, LESTER, Graduate Student, University of Pennsylvania.
GRIMM, MADELON R., Graduate Student in Botany, Cornell University.
GROSS, PAUL RANDOLPH, Graduate Student, University of Pennsylvania.
HALABAN, ATIDA, Graduate Student, University of Pennsylvania.
HARDING, DRUSILLA, Research Assistant, University of Pennsylvania.
HASTINGS, JOHN WOODLAND, Graduate Student, Princeton University.
HAY, ELIZABETH D., Medical Student, Johns Hopkins Medical School.
HILL, RUTH F., AEC Fellow in Biophysics, Columbia University.
HODGE, ALAN JOHN, Research Assistant, Massachusetts Institute of Technology.
JAMES, THOMAS W., University of Missouri.
HONEGGER, CAROL M., Instructor, Temple University.
HUEBSCHMAN, CHARLES A., Graduate Student, Columbia University.
KATZ, MICHAEL, Graduate Student, University of Pennsylvania.
KELLY, JOHN W., Graduate Student, University of Pennsylvania.
KRAUSE, LEONARD A., Student Investigator, University of Connecticut.
MALAMED, SASHA, Assistant Instructor, University of Pennsylvania.
MCCULLOCH, DAVID, AEC Fellow in Biology, Massachusetts Institute of Technology.
MCINTYRE, PATRICIA A., Johns Hopkins Medical School.
MEYER, HERTHA, Head of Tissue Culture Lab., Institute of Biophysics of University of Brazil.
MICHELINI, FRANCIS J., Research Fellow, University of Delaware.
MITCHELL, CONSTANCE JANE, University of Delaware.
NEILL, WILLIAM A., Student, Amherst College.
PASSANO, LEONARD MAGRUDER, Laboratory Assistant, Yale University.
PROCTOR, NATHANIEL K., Student, University of Pennsylvania.
RUSK, MALCOLM L., Graduate Student in Zoology, Indiana University.
SAN PIETRO, ANTHONY, Graduate Student, Columbia University.
SCLUFER, EVELYN, Graduate Student, Bryn Mawr College.
SLANE, GERTRUDE M., Graduate Student, St. Louis University.
STEWART, PETER A., Teaching Assistant, University of Minnesota.
STIEGLITZ, ALICE ANNE, Student, University of Pennsylvania.
SULLIVAN, ROBERT JAMES, Instructor, Fordham University.
TWEEDELL, KENYON S., Teaching Assistant, University of Illinois.
WEBER, PATRICIA R., Graduate Fellow, St. Louis University.

Research Assistants, 1950

ALLEN, FRANK L., University of Chicago.
ALLAMAN, LOREN E., Northwestern University.
ANDERSON, NORMAN G., Duke University.
BRAGANCA, BEATRIZ M., McGill University.
BRYAN, ELIZA R., Columbia University.
BRYAN, GEORGE C., Columbia University.
CHATTERJEE, J., Washington University Medical School.
CURRIER, JEANNE, Vassar College.
CURTIS, PAUL, Oberlin College.
DAVIDSON, MARGARET ELIZABETH M., McGill University.
DEKORNFELD, THOMAS J., Georgetown Medical School.

FASS, JEROME S., Rockefeller Institute for Medical Research.
FINGERMAN, MILTON, Northwestern University.
FITCH, FRANK W., University of Chicago.
FLESHER, JAMES W., University of Chicago.
FOLEY, MARY T., Yale University.
GELEWITZ, EDMUND W., Northwestern University.
GLASS, RICHARD, Princeton University.
GROSS, SAMSON R., Columbia University.
GUYSELMAN, J. BRUCE, Northwestern University.
HOWARD, ROBERT STEARNS, Northwestern University.
HULFISH, BARBARA, University of Rochester.
JACOB, MIRIAM I., Rockefeller Institute.
JOSEPH, ROSALIE, Columbia University.
KAPLAN, ANN ESTHER, University of Pennsylvania.
KISLIUK, ROY, Columbia University.
LANDAU, JOSEPH VICTOR, New York University, Washington Square College.
LEBARON, GEORGE I., JR., Union College.
LEONARD, LAWRENCE M., Haverford College.
LICHTENSTEIN, ROBERT, Harvard Medical School.
LITT, MORTIMER, University of Rochester Medical School.
MCINTYRE, JANE H., Bryn Mawr College.
MITCHELL, RUTH, Columbia University.
MOE, ROBERT, Fordham University.
NATHANSON, NEAL, Harvard Medical School.
ORSKI, BARBARA M., Harvard Medical School.
PADAWER, JACQUES, New York University.
PRESCOTT, DAVID M., Yale University.
ROSEN, SAUL W., Northwestern University.
SANDEEN, MURIEL, Northwestern University.
SCHNEIDER, LILLIAN K., Columbia University.
STEINBERG, MAYNARD, Polytechnic Institute of Brooklyn.
STEPHENS, GROVER C., Northwestern University.
TAMM, OLGA, American Geographical Society of New York.
TANENBAUM, STUART, Columbia University.
TIETZE, FRANK, Duke University.
WEBB, H. MARGUERITE, Northwestern University.
WILSON, IRWIN B., Columbia University.
WYCIK, EDNA, Peter Bent Brigham Hospital.

Library Readers, 1950

BARTLETT, JAMES H., Professor of Physics, University of Illinois.
BEVELANDER, GERRIT, Professor of Histology, New York University.
BODANSKY, OSCAR, Head, Clinical Biochemistry, Memorial Hospital.
BURIAN, RICHARD, Retired Professor of Physiology, 262 Kent Street, Brookline, Mass.
CANTONI, G. L., Associate Professor in Pharmacology, Western Reserve University.
CASE, JAMES F., Graduate Student, Johns Hopkins University.
CAVALIERI, LIEBE F., Research Associate, Sloan-Kettering Institute.
CLARK, ELIOT R., Professor of Anatomy *Emeritus*, University of Pennsylvania.
CSEPEL, MARTHA DE, Laboratory Technician, Rockefeller Institute for Medical Research.
DELAMATER, EDWARD D., Associate Research Professor, University of Pennsylvania.
EICHEL, BERTRAM, Assistant Research Specialist, Rutgers University.
EICHEL, HERBERT J., Teaching Assistant in Physiology, Rutgers University.
FRIES, E. F. B., Assistant Professor, The City College of New York.
FRIEDLER, GLADYS, Graduate Student, University of Pennsylvania.
FROEHLICH, ALFRED, Associate, May Institute for Medical Research.
FREUND, JULES, Public Health Research Institute of the City of New York.
GABRIEL, MORDECAI, Assistant Professor of Biology, Brooklyn College.

GOFF, RICHARD A., Assistant Professor of Zoology, University of Oklahoma.
GOULD, HARLEY N., Professor of Biology, Newcomb College of Tulane University.
GUDERNATSCH, FREDERICK, 41 Fifth Avenue, New York 3, New York.
GUREWICH, VLADIMIR, Assistant Visiting Physician, Bellevue Hospital.
HEROUX, OLIVIER, Graduate Student, Laval University.
HUGHES, ARTHUR, Research Fellow, Strangeways Research Laboratory, Cambridge, England.
KAAN, HELEN W., Research Associate, National Research Council.
KABAT, ELVIN A., Associate Professor of Bacteriology, Columbia University.
KEOSIAN, JOHN, Professor of Biology, Rutgers University.
KRASNOW, FRANCES, Head, Department of Research, The Guggenheim Dental Foundation.
KUNITZ, MOSES, Member, Rockefeller Institute for Medical Research.
LANSING, ALBERT I., Washington University, School of Medicine.
LEVINE, CELIA, Graduate Student, Columbia University.
LING, GILBERT, Instructor, Johns Hopkins Medical School.
LOEWI, OTTO, Research Professor, New York University College of Medicine.
MCCABE, MARCIA, Mount Holyoke College.
MCDONALD, SISTER ELIZABETH SETON, College of Mount St. Joseph, Ohio.
NADEAU, L. V., Graduate Student in Philosophy, College of St. Thomas Aquinas.
PERSON, PHILIP, Research Associate, Rutgers University.
QUASTEL, J. H., Professor of Biochemistry, McGill University.
REINER, JOHN R., Senior Enzyme Chemist, Columbia University.
ROSENTHAL, THEODORE B., Research Associate, Washington University.
ROWELL, L. S., Associate Professor in Zoology, University of Vermont.
ROYS, CHESTER, University of Iowa.
SCHALLEK, WILLIAM B., Hoffmann-LaRoche, Inc.
SULKIN, S. EDWARD, Southwestern Medical School, University of Texas.
TAUSSKY, H. H., Research Fellow, Cornell Medical College.
WAINIO, WALTER W., Associate Research Specialist, Rutgers University.
WEINSTEIN, MARVIN J., Research Assistant, Squibb Institute for Medical Research.
YNTEMA, CHESTER L., Professor of Anatomy, State University of New York, Medical Center.
WOOD, DARWIN L., AEC Fellow, Ohio State University.

Students, 1950

BOTANY

BARNES, ALBERT M., Bowdoin College.
DAVIS, LURETTA, Student, Harvard-Radcliffe.
FEDERBUSH, SYLVIA, The City College of New York.
HANSEN, JØRGEN B., University of Copenhagen.
JAMES, JACQUELINE, Student, Vassar College.
JOHNSON, NANCY G., Teaching Assistant, University of Wisconsin.
KEYES, CAROLYN, Wheaton College.
KRAUSS, ROBERT W., Graduate Assistant, University of Maryland.
LEMESURIER, MARGARET G., Student, McGill University.
MILSTREY, ROGER A., Drew University.
NIEDERGANG, ETHEL, Graduate Student, Brooklyn College.
POWERS, ANN W., Wellesley College.
ROSS, ELIZABETH G., Elmira College.
SILBERGER, JULIUS, Student, Harvard College.
STROUT, PHYLLIS M., Student, Goucher College.

EMBRYOLOGY

ANDERSON, IRENE L., Graduate Student, Brown University.
BABBOTT, ELIZABETH, Student, Connecticut College.
BERNSTEIN, PAUL W., Instructor of Biology, Wesleyan University.
CARPÉ, LESTRA M., Sarah Lawrence College.
DUNNEBACKE, THELMA H., Instructor, Smith College.
FEIGHTNER, LAWRENCE E., University of Illinois.

FOWLER, IRA, Student, Louisiana State University.
GAMERO-REYES, ALONSO, Instructor of Zoology, Instituto Pedagogico, Venezuela.
GRAVETT, HOWARD L., Associate Professor, Texas A. and M.
GREENGARD, PAUL, Student, Johns Hopkins University.
JACOBSON, EUGENE D., Undergraduate, Wesleyan University.
KONIGSBERG, IRWIN R., Junior Instructor, Johns Hopkins University.
LANSCHE, JAMES M., Student, Washington University.
LEAVITT, EARLE E., Instructor, Norwich University.
LINDBERG, ROBERT G., Teaching Assistant, University of California, Los Angeles.
MABEL, JUDITH, Goucher College.
MAGNER, ARDYS, Duke University.
McCULLOUGH, KIRK W., Instructor, Washington and Jefferson College.
McKIBBEN, JULIET, Assistant Professor of Biology, Carnegie Institute of Technology.
NEFF, RUTH H., Instructor, University of Missouri.
PEPPER, MAX P., Amherst College.
RENZI, ALFRED A., Research Assistant, Syracuse University.
SEXTON, OWEN J., Oberlin College.
SMALL, JEAN ELIZABETH, Student, University of Massachusetts.
SMITHBERG, MORRIS, Graduate Student, University of Rochester.
SPIROFF, BORIS E. N., Graduate Teaching Assistant, Northwestern University.
VAN BREEMEN, VERNE L., Research Assistant, State University of Iowa.
VOGEL, PHILIP H., Graduate Student, Loyola University.
VOLZ, RUTH, Drew University.

PHYSIOLOGY

BAIRD, JOAN AUDREY, Graduate Assistant, Syracuse University.
BURLINGTON, HAROLD, Graduate Assistant, Syracuse University.
CHIN, MARY B., Teaching Fellow, University of Michigan.
EHRMANN, ROBERT L., Johns Hopkins University Medical School.
FRIEDMAN, STANLEY, Graduate Student, Johns Hopkins University.
GLADNER, JULES A., Research Assistant, Duke University School of Medicine.
HERBERT, EDWARD, Student, University of Pennsylvania.
KARPLUS, MARTIN, Student, Harvard College.
KELLER, JOHN G. J., Graduate Fellow, St. Louis University.
KOMINZ, DAVID R., Post-doctoral Fellow, Harvard University.
LILIEN, OTTO M., Jefferson Medical College.
MARSHALL, GERALD W., University of California, Los Angeles.
MEDILL, MARY A., University of Pennsylvania.
McMASTER, RACHEL D., Graduate Student, Columbia University.
MILLER, DANIEL G., Rockefeller Institute.
MILLER, TILFORD D., Amherst College.
NEFF, ROBERT J., Research Associate, University of Missouri.
NEUKOM, BETTY JANE, Chemical Technician, University of Colorado Medical Center.
PRAGLIN, JULIUS, Assistant, University of Illinois.
REICHERT, ERIDA R., Johns Hopkins Medical School.
ROBINSON, CHARLES V., Research Associate, Harvard Medical School.
SCHNEIDER, ROSE MARY, Graduate Student, New York University.
SHAW, EVELYN S., Instructor, American Museum of Natural History.
STALLWORTHY, WILSON B., Instructor in Zoology, University of Maine.
STROMINGER, JACK L., Washington Medical School.
TOWNES, PHILIP L., Graduate Student, University of Rochester.
deVILLAFRANCA, GEORGE W., Student, Yale University.

INVERTEBRATE ZOOLOGY

ANDERSON, EVERETT, Graduate Student, Fisk University.
ANDERSON, JANET L., Teaching Assistant, University of Michigan.
BELMONTE, REV. ROCH G., Instructor in Biology, Fordham University.
BERNE, BERNARD, Assistant Instructor, University of Illinois.
BERNHARDT, EVA G., Associate Professor of Biology, Huntington College.

Birkner, Marion L., University of Illinois.
Bowman, Marjorie C., Graduate Student, University of Toronto.
Campbell, Mildred A., Smith College.
Conover, Robert J., Oberlin College.
Cory, B. Lawrence, Graduate Student, Notre Dame University.
Cotter, William B., Jr., Graduate Student, Wesleyan University.
Dossel, William E., Graduate Assistant, Marquette University.
Ede, Donald A., Assistant, Northwestern University.
Erk, Frank C., Johns Hopkins University.
Etges, Frank J., Graduate Assistant, New York University.
Forsthoefel, Rev. Paulinus F., Graduate Student, Ohio State University.
Gustafson, John A., Assistant, Cornell University.
Haddow, Suzanne, Student, Skidmore College.
Hodge, Mary H., Wilson College.
Hugghins, Ernest J., Teaching Assistant, University of Illinois.
Hunter, Robert L., University of Michigan.
Hyland, Kerwin E., Jr., Graduate Student, Duke University.
Imbrie, John, Graduate Student, Yale University.
Jenerick, Howard P., Public Health Service Fellow, University of Chicago.
Johnson, Harry M., Student, Cornell University.
Kaye, Alvin M., Senior at Columbia College, Columbia University.
Kelley, Joan M., Vassar College.
Krause, Leonard A., Student, University of Connecticut.
Lathrop, Merle Inez, Instructor, Smith College.
Levin, Norman L., Student, University of Illinois.
Lieb, Rev. Joel R., Graduate Student, St. Louis University.
Lippitt, Ruth H., Student, Wellesley College.
Lyon, John B., Jr., Hamilton College.
MacMillan, Shirley A., Student, Oberlin College.
Mann, David E., Jr., Purdue University.
Maynard, Donald M., Student, Harvard University.
McCashland, Benjamin W., Instructor of Physiology, University of Nebraska.
McDermott, William M., Tufts College.
Newman, Ellen, Radcliffe College.
Rolle, Gloria K., Laboratory Technician, New York University.
Saunders, Richard L., University of Massachusetts.
Scheltema, Rudolf S., George Washington University.
Seale, Joanne K., Pennsylvania College for Women.
Shaw, Edward I., Graduate Assistant in Zoology, University of Missouri.
Stahl, Walter, Massachusetts Institute of Technology.
Tamar, Henry, Graduate Student, New York University.
Tassoni, Joseph P., Assistant in Biology, New York University.
Timm, Rev. Richard W., Graduate Student, Catholic University.
Trefz, Shirley, Temple University.
Urso, Paul, Jr., Student, St. Francis College.
Van Aken, Mary C., Graduate Student, Yale University.
Vernberg, Frank J., Graduate Student, Purdue University.
Vernberg, Winona M., Graduate Student, Purdue University.
Walsh, Winifred, Student, Hunter College.
Weinert, Sanford D., Graduate Student, Harvard University.

4. The Lalor Fellows, 1950

James Danielli, King's College, London, England.
Seymour S. Cohen, University of Pennsylvania.
William P. Jacobs, Princeton University.
Sally M. Kelly, Vassar College.
Alan H. Mehler, University of Chicago.
Stuart Smith, University of Colorado Medical School.

5. Tabular View of Attendance, 1946–1950

	1946	*1947*	*1948*	*1949*	*1950*
Investigators—Total	267	299	326	344	338
Independent	175	182	183	193	198
Under instruction	29	36	42	52	43
Library Readers	38	36	50	55	48
Research Assistants	25	45			49
Students—Total	122	131	123	128	126
Zoology	57	55	54	55	55
Embryology	30	33	29	31	29
Physiology	26	26	25	27	27
Botany	9	17	15	15	15
Total Attendance	389	430	449	472	444
Less persons registered as both students and investigators		2	6	2	
	389	*428*	*443*	*470*	*444*
Institutions Represented—Total	*141*	*148*	*158*	*155*	*156*
By Investigators	102	114	117	114	114
By Students	56	56	68	68	67
Schools and Academies Represented					
By Investigators	2	1	1	1	2
By Students		1			
Foreign Institutions Represented					
By Investigators	7	7	8	6	6
By Students	5	3	4	3	2

6. Subscribing and Cooperating Institutions, 1950

Subscribing Institutions

Children's Hospital of Philadelphia
Elmira College
Georgetown University Medical School
Hahnemann Medical School
Haskins Laboratories
Indiana University
Marquette University
Morgan State College
National Research Council
Pennsylvania College for Women
Public Health Research Institute of New York City
Radcliffe College
Rutgers University
St. Francis College
Southwestern Medical College
Tulane University School of Medicine
University of Miami
University of New Hampshire
University of Rochester
University of Wisconsin
Vanderbilt University School of Medicine
Wellesley College
Wheaton College
Wilson College

Cooperating Institutions

Amherst College
Argonne National Laboratory
Brooklyn College
Brown University
Bryn Mawr College
College of Mt. St. Joseph-on-the-Ohio
College of Physicians and Surgeons
Columbia University
Cornell University
Duke University
Emory University
Fordham University
Goucher College
Harvard University
Harvard University Medical School
Hunter College
Institute for Muscle Research
Johns Hopkins University
Johns Hopkins University Medical School
Eli Lilly and Company

Madison Foundation
Massachusetts Institute of Technology
Mount Sinai Hospital
National Cancer Institute
New York University College of Medicine
New York University, Heights
New York University, Washington Square College
North Carolina State College
Northwestern University
Oberlin College
Princeton University
Rockefeller Institute for Medical Research
St. Louis University
Sloan-Kettering Institute
State University of Iowa
Temple University
Tufts College Medical School
Union College
University of Chicago
University of Delaware
University of Illinois
University of Kansas
University of Massachusetts
University of Maryland School of Medicine
University of Michigan
University of Minnesota
University of Missouri
University of Oklahoma
University of Pennsylvania Medical School
University of Pennsylvania
University of Vermont Medical School
University of Virginia
Vassar College
Washington University
Wesleyan University
Western Reserve University
Yale University

7. Evening Lectures

The Friday Evening Lectures, 1950

Friday, June 30
Dr. A. E. Szent-Györgyi ..... "Life and Muscle."
Friday, July 7
Dr. Arthur W. Pollister ..... "Studies in Cytology."
Friday, July 14
Dr. Carroll M. Williams ..... "A Hormonal-Enzymatic Mechanism for the Control of Growth and Differentiation in the Cecropia Silkworm."
Friday, July 21
Dr. James Danielli ..... "The Phosphatase of Cell Nuclei."
Friday, July 28
Dr. H. H. Plough ..... "Radiation Induced Mutations in *Salmonella typhimurium* and Their Genetic Implications."
Friday, August 4
Dr. John Runnström ..... "Some Aspects of the Developmental Physiology of the Sea Urchin."
Friday, August 11
Dr. Chester L. Yntema ..... "Induction and Differentiation of the Ear."
Friday, August 18
Dr. Robert F. Pitts ..... "Renal Control of Acid Base Balance."
Friday, August 25
Dr. Clarence Cottam ..... "Wildlife Management in Relation to America's River Development Program."

Other Lectures

Wednesday, August 23
Dr. D. O. Jordan ..... "Recent Studies on Desoxyribosenucleic Acid."
Dr. A. G. Ogston ..... "Experiments on Hyaluronic Acid."

8. Seminars, 1950

July 5

Anita Zorzoli ..... "Alkaline Phosphatase Activity in Normal and Scorbutic Bones."

S. J. Cooperstein ..... "The Reduction of Janus Green by Isolated Enzyme Systems."

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Milton Levy ..... "Denaturation of Ricin as Affected by pH and Temperature."

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Charles B. Metz ..... "Amino Acids and Peptides as Adjuvants in Agglutination of Starfish Sperm by Fertilizin."

Albert Tyler ..... "Extension of Functional Life Span of Spermatozoa by Amino Acids and Peptides."

J. Runnström ..... "The Effect of Ca and Jelly Coat Substance on the Agglutination and Respiration of Sea Urchin Spermatozoa."

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M. H. Jacobs and H. N. Glassman .... "Permeability of the Erythrocyte at Different Temperatures."

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Emmanuel Fauré-Fremiet ..... "Feeding Reactions of Some Predator Ciliates."

Paul A. Nicoll ..... "Subcutaneous Blood Flow in the Bat's Wing."

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WOLF VISHNIAC ..... "Ionic Balance Required by Some Marine Microorganisms and the Effect of Quaternary Ammonium Ions."

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NORMAN MILLOTT ..... "The Reactions to Shade and the Pigmentation of the Sea Urchin *Diadema antillarum*."

HAROLD F. BLUM AND MARGIE R. MATHEWS ..... "Photorecovery after Ultraviolet Radiation, in Amphibian Larvae."

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K. C. ATWOOD ..... "The Homology Pattern of Induced Lethal Mutations in *Neurospora crassa*."

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PHYSIOLOGICAL STUDIES OF DIFFERENTIATION IN MYTILUS EDULIS. I. THE OXYGEN UPTAKE OF ISOLATED BLASTOMERES AND POLAR LOBES[1]

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The classic experiments of Wilson (1904), Conklin (1905) and others have demonstrated that the eggs and embryos of many invertebrate animals contain regions of cytoplasm which are determined as to their subsequent fate. A precocious differentiation may even precede cleavage of the eggs, as in some annelids, molluscs, and ascidians. The cytoplasmic regions of these so-called mosiac eggs have been described variously as containing "organ forming" substances or of being chemo-differentiated, but as yet there has been slight progress in defining these mosaic regions in terms of specific physicochemical differences.

Spek (1934) attempted to demonstrate regional cytoplasmic differences by means of vital dyes and concluded on the basis of differential vital staining that a pH gradient existed along the polar axis of various mosaic eggs. Ries (1937, 1939) compared mosaic and regulative eggs by the use of cytochemical techniques. Tests for ascorbic acid, glutathione, benzidine peroxidase, and indophenol oxidase were used, in addition to measurements of rH_2 by means of oxidation-reduction dyes. In some mosaic eggs a localization of these substances or a difference in redox potential could be demonstrated, although the significance of these in the process of differentiation was unclear. Similar investigations on mosaic eggs have been carried out and extended by Reverberi and Pitotti (1940), Pitotti (1947) and Urbani and Mistruzzi (1947). Cytochemical tests have also been used to demonstrate localization of enzymes in mosaic eggs by Lehmann (1948) and Raven (1948).

Although cytochemical techniques have yielded valuable information regarding the chemical heterogeneity of mosaic eggs, they have the disadvantages of being limited in scope as well as being mainly qualitative. Also considerable controversy exists as to the validity of some of the cytochemical techniques (Brachet, 1947). It is apparent that the most desirable approach to this problem would be to carry out direct microchemical analyses and physiological studies of the various cytoplasmic regions. It was with this intention that the present investigation was undertaken.

Owing to the small size of mosaic eggs, it would be difficult to obtain, by cutting the egg into fragments, sufficient amounts of cytoplasm for microchemical studies. It is known, however, that in several mosaic eggs the first cleavage plane normally separates cytoplasmic regions with different presumptive fates. This is particularly striking in the eggs which form polar lobes such as those of Dentalium, Ilyanassa

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and Myzostoma. It seemed that a profitable approach to the problem of cytoplasmic localization would be to take advantage of this natural segregation of cytoplasmic areas and to develop methods for large scale separation of blastomeres in such a form.

The eggs and embryos of *Mytilus edulis* have proven to be particularly suitable for this purpose. Field (1921) has described the early development of *M. edulis* in detail. The first cleavages are similar to those of Dentalium (Wilson, 1904); a polar lobe forms at the vegetal pole of the fertilized egg and is subsequently incorporated into the CD blastomere. Prior to the next cleavage a second polar lobe forms which fuses with the D cell. The first cleavage blastomeres are unequal in size and therefore may be readily recognized.

Investigations on the development of isolated blastomeres and eggs lacking the polar lobes indicate that a typical cytoplasmic localization exists in the Mytilus egg. The polar lobe contains factors which are necessary for the normal development of the larval form and particularly for an apical tuft (to be published).

As a prerequisite for physiological and microchemical studies, it was necessary to develop methods for separating the blastomeres of early cleavage stages of Mytilus. Even on a small scale this is ordinarily difficult, owing to a tough protective membrane surrounding the eggs and an intercellular cement binding the blastomeres together. Fortunately it was discovered that sperm extracts contain lysins which dissolve the enclosing membranes as well as the intercellular cement (Berg, 1950). The use of these lysins permits large scale separation of the blastomeres which subsequently may be used for physiological studies. A method has also been discovered for obtaining large numbers of isolated polar lobes which permits investigations to be carried out directly on the polar lobe itself.

The present report, dealing with the respiration of isolated blastomeres and polar lobes of Mytilus, was undertaken in view of the many attempts in the past to correlate embryonic differentiation with metabolic gradients. Child (1941), for example, has for many years postulated physiological gradients or "respiratory gradients" as controlling factors of differentiation. Ries (1942) also suggests that there is a coupling of oxidation intensity and the oxidase content of regions of mosiac eggs. These conclusions, however, have been based on the results of cytochemical studies, whereas the present techniques permit direct quantitative measurements of the respiratory intensity of different regions of the egg. A preliminary report of this work has recently appeared (Berg and Kutsky, 1950).

Methods

Mytilus edulis were collected from the San Francisco Bay and stored "dry" in a refrigerator at 4° C. Eggs and sperm were obtained by placing animals in separate bowls of sea water and allowing natural spawning to occur. Although spawning occurs up to one week after storage of the animals at low temperatures, as a rule they were used within the first few days to avoid a progressive deterioration of the eggs.

The procedure for separation of the first cleavage blastomeres is based upon the discovery of lysins in sperm extracts. As previously reported (Berg, 1950), sperm extracts prepared by freezing and thawing of a concentrated sperm suspension contain lysins which dissolve the membrane enclosing the egg and also the inter-

cellular cement binding the blastomeres together. For the present experiments it was convenient to prepare sperm extracts from the testes of *M. californianus* since considerably greater amounts of sperm may be obtained from this species. Several liters of a concentrated sperm suspension were prepared by homogenizing the testes of several dozen males. This was frozen, centrifuged, and the supernatant measured into 10-ml. vials which were stored at −20° C.

Separation of the first cleavage blastomeres was carried out using sperm extract alone or by a combination of sperm extract and calcium-free sea water. With the former method the eggs were fertilized and approximately five minutes before cleavage the excess sea water was pipetted off and 5–10 ml. of sperm extract added. Dissolution of the egg membranes occurred in a few minutes and, as described previously (Berg, 1950), on completion of the first cleavage the blastomeres became partially or completely separated. It was usually necessary to draw the eggs gently in and out of a pipet to increase the percentage of complete separation. Calcium-free sea water also dissolves or prevents the formation of intercellular cement and may be used for blastomere separation. With this method the membranes were removed by means of the sperm extract shortly after fertilization and the eggs were then transferred through several dishes of calcium-free sea water.

The use of sperm extract alone is preferred in some instances since the blastomeres in calcium-free sea water are more fragile and the agitation necessary to ensure complete separation often causes cytolysis. Both methods, however, have been used satisfactorily. After separation, the blastomeres were transferred through several dishes of normal sea water.

Isolated polar lobes were obtained by subjecting membraneless eggs to $MgCl_2$ solutions at the time of polar lobe formation, a procedure which causes a number of the polar lobes to constrict off rather than fuse with the CD cells. Moderate agitation increases the percentage of isolated polar lobes. Since there is considerable variability in the success of this method and the factors involved have not been studied in detail, a fuller report of this method will be published at a later date.

The oxygen uptake of whole eggs, isolated blastomeres, and polar lobes was measured with the Cartesian diver ultramicrorespirometer. Divers were constructed from Pyrex glass according to a design described by Zeuthen (1950), in which the part of the diver containing the biological material is plugged with a hollow glass stopper. Air volumes of the divers ranged from 0.4 μl. to 1.1 μl. and the liquid charge amounted to about two-thirds the size of the air bubble. In paired experiments divers with approximately the same volume were nearly always used since there was a tendency for larger divers to give slightly higher oxygen uptake values. As reported by Zeuthen, these divers are practically gas tight. Blank tests in which pure oxygen or nitrogen was introduced as the bubble resulted in a drift of the equilibrium volume of about 0.1 cm. manometer pressure per hour (after an initial equilibration period).

The flotation vessels were filled with alkaline sodium chloride solution isotonic with sea water. Equilibrium pressures, as determined by raising the diver slightly off the bottom of the flotation chamber, were measured every 10 to 20 minutes depending upon the type of experiment. In nearly all experiments the number of cells introduced into the diver was chosen so as to cause a manometer change of 10–15 cm. per hour.

Immediately after placing a loaded diver in the flotation chamber, the equilibrium pressure decreases rapidly at first and then more slowly. This is probably due to equilibration taking place between the liquid and gas phases of the diver charge. Accordingly actual measurements of oxygen uptake were not begun until after a half hour equilibration period. The error due to a residual drift becomes negligible (less than 0.5 cm. per hour) after about 30 minutes. All experiments were carried out at 20° C.

Oxygen uptake was calculated in the usual manner from the volume of the bubble at equilibrium pressure, the change of the equilibrium pressure per unit time, and the number of cells. Corrections were made for the decrease in oxygen tension in the liquid charge and also for the manometer pressure during the period of measurement.

The Oxygen Uptake of Isolated Blastomeres

AB and CD cells were separated from one another by either of the previously described methods and then transferred through several dishes of sea water. Since AB cells average about 41 μ in diameter and CD cells about 51 μ, they can be distinguished under a low power microscope. The dish from which the blastomeres were to be counted was kept at 10°–15° C. in order to delay cleavage, as it is somewhat easier to distinguish the two types of cells prior to their first cleavage. In each experiment 75 CD cells were selected under the microscope with a braking pipet and introduced into the diver by inserting the tip of the pipet into the diver and slowly expelling the cells so that they settled to the bottom. Loading of the diver was done under a dissecting microscope with the diver held in a fixed position on a small glass plate. One hundred fifty AB cells were selected and loaded similarly into another diver. Air bubbles were measured with a calibrated braking pipet and introduced into the respective divers. The sea water above the air bubbles was then displaced with an alkaline sodium chloride solution, the divers stoppered, placed in the flotation chambers and allowed to equilibrate for approximately 30 minutes.

After loading the divers, the diameters of representative samples of AB and CD cells (usually about 20 to 30 each) were determined with an ocular micrometer. The measured blastomeres were selected from the same batch as that used for the respiration experiment.

The time necessary for counting the cells, loading the divers, etc., caused a delay such that actual oxygen uptake measurements could not begin until nearly three hours after fertilization. At this time the blastomeres had undergone cleavage and were all at least in the two-cell stage. Respiration was measured for an 80-minute period in which time several more cleavages occurred and the embryos consisted of eight or more cells. It might be pointed out here that the cleavage rates of the AB and CD cells are nearly identical during their early development (Fig. 4).

In initial experiments it became apparent that due to biological variability and experimental errors, it would be necessary to carry out a number of experiments and apply a statistical test. To facilitate the statistical analysis paired experiments were carried out, *i.e.*, AB and CD cells were obtained from the same batch of eggs and the oxygen uptake of these determined simultaneously. The data for 12 paired experiments are listed in Table I. Oxygen uptake was first calculated, using the equilibrium volume of the bubble, change in manometer pressure for the period,

number of cells, etc., and with the appropriate corrections expressed as μl. O_2 uptake per hour per embryo. Since the amount of cytoplasm in the AB and CD embryo differs considerably, it was decided to express oxygen uptake in terms of unit amount of cytoplasm. The above figures were therefore divided by the average initial volume of the cells for the particular experiment and final comparative values could then be expressed as μl. oxygen uptake/hr./mm.^{3} cytoplasm.

The average of 12 experiments on the respiration of AB embryos, as shown in Table I, is $0.90 \pm .032$ μl. O_2/hr./mm.^{3} cytoplasm, as compared with $0.78 \pm .027$ for

TABLE I

Comparison of oxygen uptake of isolated AB and CD embryos for an 80-minute interval three hours after fertilization

Experiment No.	Oxygen uptake of AB embryos 10^{-5} μl. O_2/hr./AB embryo	Average volume of AB cells 10^{-5} mm.^{3}	Oxygen uptake of AB embryos 10^{-1} μl. O_2/hr./mm.^{3} AB cytoplasm	Oxygen uptake of CD embryos 10^{-5} μl. O_2/hr./CD embryo	Average volume of CD cells 10^{-5} mm.^{3}	Oxygen uptake of CD embryos 10^{-1} μl. O_2/hr./mm.^{3} CD cytoplasm
1	3.1	3.95	7.8	4.8	7.54	6.4
3	2.9	3.27	8.9	4.9	6.62	7.4
4	3.0	3.46	8.7	5.2	7.10	7.3
6	3.2	3.40	9.4	5.5	6.62	8.3
8	3.5	4.24	8.3	6.7	8.52	7.9
9	3.0	3.82	7.9	5.4	7.62	7.1
10	3.0	3.58	8.4	4.8	7.35	6.5
11	3.5	3.66*	9.6	6.9	7.35*	9.4
12	2.8	3.66*	7.7	5.8	7.35*	7.9
13	3.8	3.95	9.6	6.0	7.54	8.0
14	3.5	3.52	9.9	5.9	7.10	8.3
15	4.0	3.46	11.5	6.3	6.80	9.3
	Average		$9.0 \pm .32$ S.E.			$7.8 \pm .27$ S.E. (13% lower than respiratory rate of AB embryos). $t = 5.6$, sig. at 1% level

* Diameters not measured. Average of all experiments used.

the corresponding CD embryos. The difference of 13 per cent is significant at the 1 per cent level, and it may be concluded from this that the respiration of CD cytoplasm is significantly lower than that of AB cytoplasm.

In addition to the above experiments three others were carried out which were not paired, *i.e.*, the AB and CD cells did not come from eggs of the same female. If these are included with the data of Table I, the rate of O_2 uptake of the CD embryos is found to be 15 per cent less than that of AB embryos. Statistical treatment of this unpaired series indicates a difference significant at the 1 per cent level.

THE OXYGEN UPTAKE OF ISOLATED POLAR LOBES

The polar lobe is incorporated into the CD cell at the close of the first cleavage and one might reasonably suspect that any physiological differences existing between

the first cleavage blastomeres might be due to the polar lobe. Thus, the lower respiratory rate of the CD cells might be owing to the presence of the polar lobe material.

Polar lobes were isolated by the use of sperm extracts and $MgCl_2$ solutions, as described previously, and transferred through several dishes of normal sea water. In nine experiments 425–475 lobes were drawn up into a fine braking pipet and loaded into a diver in the same manner as described for AB and CD cells. A sample (30 to 40) of polar lobes was removed from the same dish and their diameters measured. The average diameter of isolated polar lobes is almost 30 microns which is about 10 microns less than the AB cells. AB cells were also present in the dish

TABLE II

Comparison of oxygen uptake of isolated polar lobes and whole eggs

Oxygen uptake of whole eggs during time of polar lobe formation 10^{-6} µl./hr./egg	Average volume of eggs 10^{-5} mm.^{3}	Oxygen uptake of eggs during time of polar lobe formation 10^{-1} µl. O_2/hr./mm.^{3} egg cytoplasm	Oxygen uptake of isolated polar lobes 10^{-6} µl./hr./polar lobe	Average volume of polar lobes 10^{-5} mm.^{3}	Oxygen uptake of polar lobes 10^{-1} µl. O_2/hr./mm.^{3} polar lobe cytoplasm
6.7*	12.3	5.4	5.6	1.39	4.0
6.8*	13.7	5.0	6.8	1.71	4.0
6.9*	12.6	5.5	4.8	1.56‡	3.1
6.6	12.5	5.3	8.7	1.84	4.7
7.3	12.4	5.9	6.7	1.50	4.5
7.5	12.0	6.3	5.2	1.22	4.3
6.8	12.2‡	5.6	6.3	1.56‡	4.0
6.8	11.8	5.8	4.9	1.39	3.5
6.3	12.2‡	5.2	8.1	1.41	5.7
Average		5.6 ± .13 S.E.			4.2 ± .25 S.E. (25% lower than respiratory rate of whole egg cytoplasm. t = 4.9, sig. at 1% level)

* Average of two or more experiments on the same batch of eggs.
‡ Diameters not determined. Average of all experiments used.

with isolated polar lobes and it was sometimes difficult to distinguish between the largest of the polar lobes and the smallest of the AB cells, particularly when several hundred polar lobes had to be selected rather rapidly. In almost all experiments a few AB cells were thus unavoidably included in the diver with the polar lobes. These served a useful purpose, however, since the presence of swimming AB embryos in the diver after the respiration measurements served as an indication of the validity of the test. In calculating the respiration of the polar lobes a correction was made for the respiration of these AB cells.

Actual oxygen consumption measurements were carried out for a period of at least three hours. The polar lobes were examined afterwards, and a few tests were discarded because of a marked clumping of the lobes. This, coupled with the absence of swimming AB embryos, suggested that alkali had crept past the air bubble and mixed with the sea water.

For purposes of comparison the respiration of whole eggs was determined during the time of polar lobe formation. A period of 20 minutes was chosen, beginning 60 minutes after fertilization which is approximately the time at which the polar bulge first appears. Fertilized eggs were first treated to remove the membranes and then washed with sea water. In each experiment 50 to 75 of these were loaded into a diver as rapidly as possible, so as to allow sufficient time for the diver to equilibrate. The diameters of representative eggs were then determined during the equilibration period.

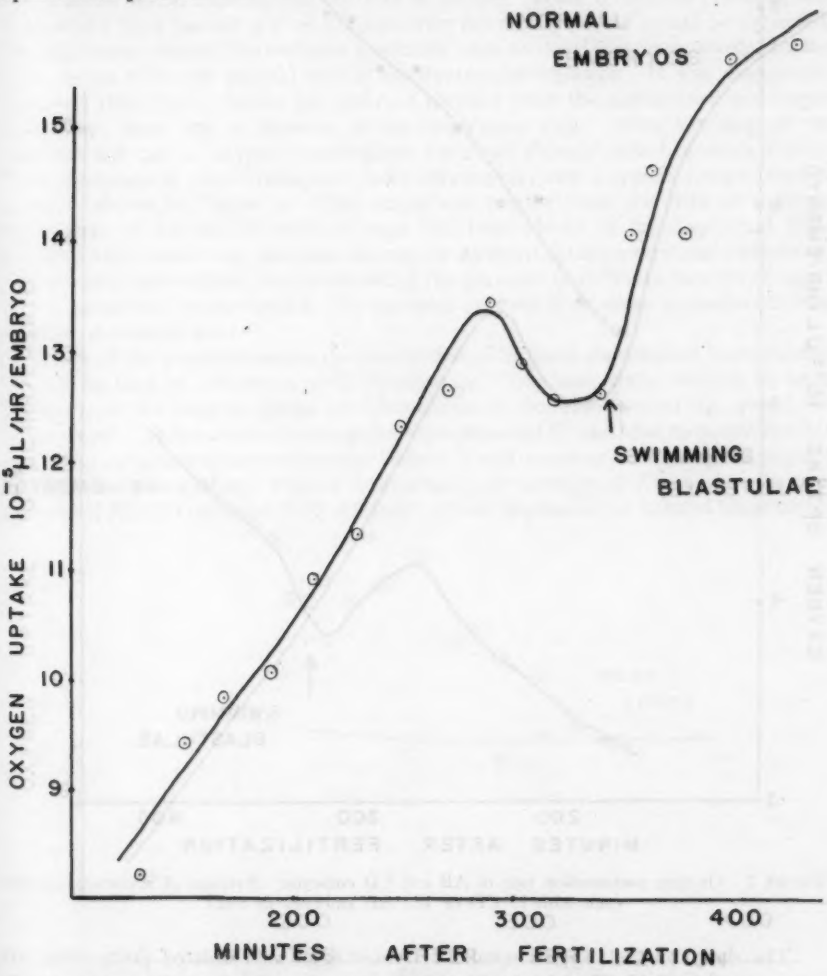

FIGURE 1. Oxygen consumption rate of normal *Mytilus edulis* embryos. 250 embryos in Cartesian diver.

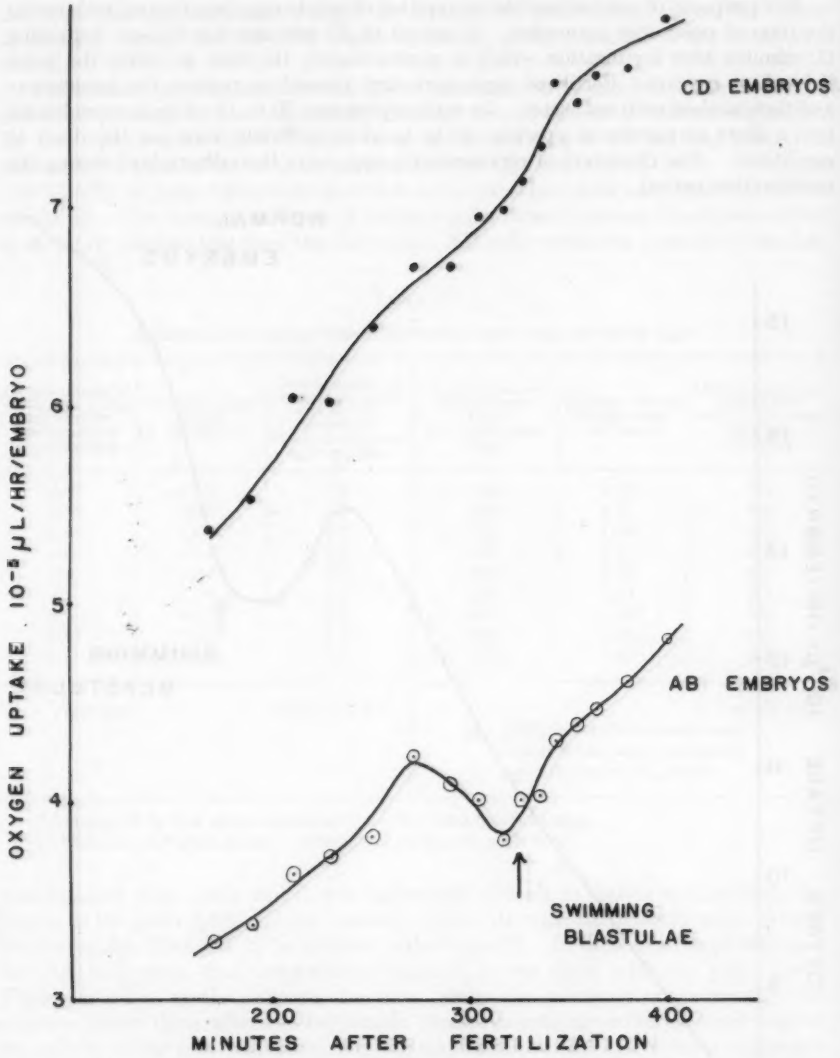

FIGURE 2. Oxygen consumption rate of AB and CD embryos. Average of seven experiments, each with 75 CD or 150 AB embryos in diver.

The data for the oxygen uptake of whole eggs and isolated polar lobes are summarized in Table II. Again, for the purpose of comparison, the oxygen uptake is expressed in terms of oxygen consumed per unit amount of cytoplasm. The average oxygen uptake of whole eggs during the time of polar lobe formation is

0.56 ± 0.013 μl. O_2/hr./mm.^{3} egg cytoplasm as compared to 0.42 ± 0.025 for the polar cytoplasm. The difference of 25 per cent is significant at the 1 per cent level (data treated as an unpaired series). It thus appears that the lower respiration of the CD cells can be explained, at least in part, on the basis of a lower oxygen consumption of the polar lobe cytoplasm.

Rate of Oxygen Uptake During Early Development

In a few initial experiments the rate of oxygen uptake of normal embryos was determined for a period of 7 or 8 hours after fertilization. As would be expected, the respiratory rate of the embryos gradually rises as development proceeds, as has been shown to be the general rule in embryonic development. It was discovered, however, that shortly before the embryos hatched from the membranes and began swimming, there was a decrease in the respiratory rate. After hatching of the blastulae the rate of oxygen consumption increased sharply and then more slowly. This phenomenon was investigated more thoroughly, and a typical oxygen uptake curve is shown in Figure 1. This curve was plotted from the data of a single experiment in which 250 fertilized eggs had been placed in the diver, and it illustrates this interesting decrease in rate of oxygen uptake preceding swimming. There was some variation in the extent of the decrease in different batches of eggs; in the particular curve figured, the decrease is somewhat more pronounced than in other determinations.

Some of the experiments on the respiration of isolated blastomeres were carried out for as long as six hours after fertilization. In these, there seemed to be a tendency for the oxygen uptake of AB embryos to decrease toward the end of the experiment. This was also investigated more thoroughly, and, due to a considerable scattering of points of the respiration curves, it was necessary to average a number of experiments together. Figure 2 illustrates the average of 7 experiments each (a total of 525 CD cells and 1050 AB cells) of the respiration of isolated blastomeres

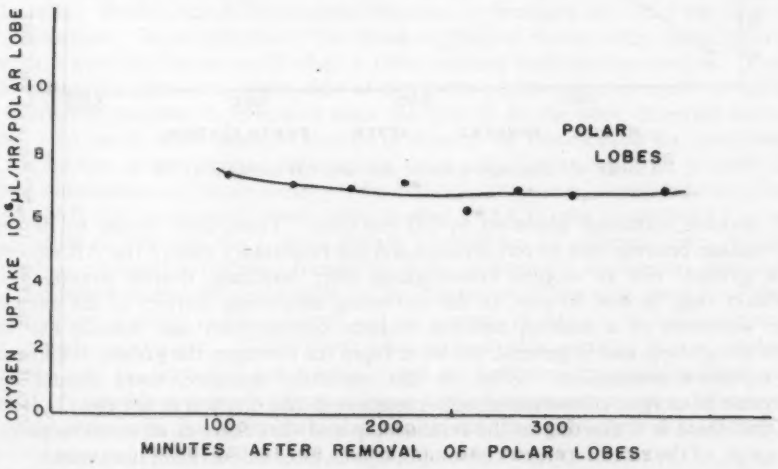

Figure 3. Oxygen consumption rate of isolated polar lobes. 467 polar lobes in diver.

up to 7 hours after fertilization. From this, it is clear that the decrease in respiration of whole embryos is due to a decrease in respiration of the derivatives of the AB cells. Whether the oxygen uptake curve of the CD embryos is a straight line or perhaps exhibits a slight decrease prior to hatching is open to question. It may be that these variations in slope are merely due to experimental errors.

It seemed possible that the above difference in respiratory rates of AB and CD embryos might be related to differential rates of cell division. Accordingly, counts were made of the number of cells composing AB and CD embryos at various stages of development (Fig. 4). It is evident that cell division in AB embryos decreases in rate and ceases at about the time the rate of oxygen consumption drops, whereas

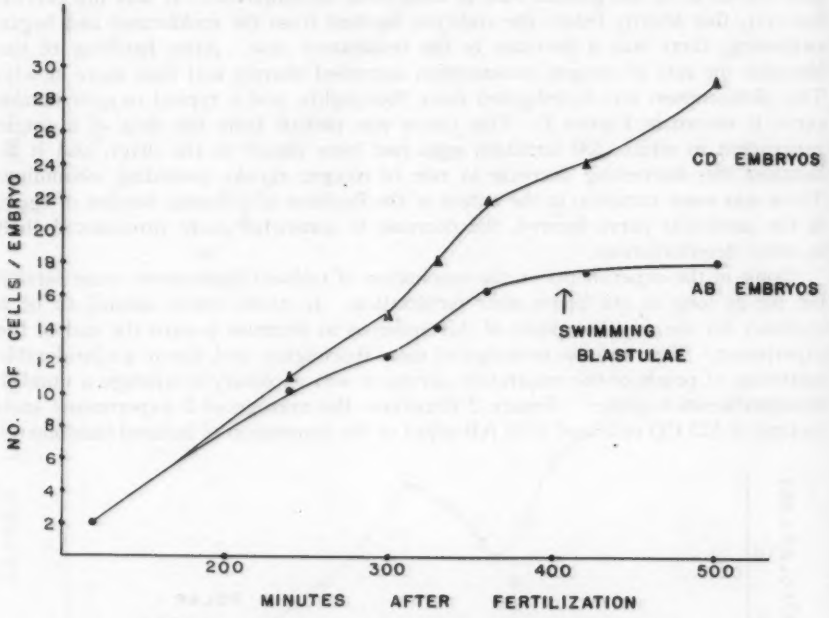

FIGURE 4. Cleavage rates of AB and CD embryos at 18° C.

cell division continues unabated in CD embryos. Thus, there seems to be some correlation between rate of cell division and the respiratory rate of the AB embryos. The gradual rise in oxygen consumption after hatching, despite arrest of cell division, may be due, in part, to the increasing swimming activity of the embryos. The existence of a relation between oxygen consumption and mitosis has long been recognized, and in general, the more rapid the cleavage, the greater the increase in oxidative metabolism. Why, in this particular instance, there should be a decrease in oxygen consumption with cessation of cell division is not clear. It may be that there is a time lag in the relationship and that there is an overshooting, so to speak, of the rate of oxygen consumption even after cell division has ceased.

The oxygen uptake rate of isolated polar lobes was followed for a period of three to four hours after their constriction from the eggs. A single experiment in which 467 polar lobes were placed in the diver is shown in Figure 3. It is significant that the polar lobe cytoplasm, at least when isolated, does not participate in the general rise in respiratory rate as exhibited by the whole eggs or isolated blastomeres. Although only a single curve is presented, five other experiments also indicated that there is no change in the respiratory rate of the isolated polar lobes.

The fact that polar lobes do not show the gradual increase in oxygen consumption indicates that the increase itself is coupled with nuclear and cytoplasmic divisions rather than being an inherent characteristic of the cytoplasm itself. Although the isolated polar lobes undergo cyclic phases of activity (ameboid activity and forming what appears to be a miniature polar lobe), no changes in oxygen consumption were detected during these periods.

Discussion

The possibility of quantitative differences in the respiration of various regions of eggs or embryos has long been a subject of speculation. Thus, in the present experiments wherein such differences have been demonstrated, it is of importance to analyze the possible sources of errors. Previous to the oxygen uptake measurements, the AB and CD cells were subjected to identical treatment, and it is unlikely that any external factors were operating to change their physiological characteristics. There is no reason to believe that a short treatment of the eggs with sperm extract or calcium-free sea water has any immediate or permanent effect on the rate of respiration.

There are, of course, errors which may be of considerable magnitude in the measurement of respiration with the Cartesian diver. Each diver has a characteristic drift of the equilibrium pressure which depends upon the size of the air volume, temperature at which it is loaded, etc. This drift is considerable at the beginning of an experiment and amounts to as much as 50 per cent of the respiration of the cells. Within about 30 minutes, however, it decreases to 1 to 5 per cent of the respiration. In order to avoid the initial large error due to drift, measurements were as a rule not begun until after a thirty-minute equilibration period. Even after this period there is a slight drift of the divers; however, this tends to cancel out in the comparative experiments since the drift is in the same direction for all divers. To avoid other possible sources of errors, the divers used for measuring the respiration of the isolated blastomeres were matched as much as possible in general dimensions and in air volume. In addition, they were alternated, the diver used for AB cells in one experiment being loaded with CD cells in the next.

In order to compare the oxygen uptake of embryos of different sizes, it was necessary to express the respiration in terms of unit amount of cytoplasm as calculated from the initial diameters of the cells. The diameters of the cells were measured with an accuracy of one or two microns and the average of 20 or more measurements used in each experiment. While there is an error in this average, it is a random error and with the statistical test used it would not influence the significance of the results. A possible source of systematic error in these measurements is a differential flattening of the cells during the time of measurement; however, there is no evidence that this occurs.

The respiratory rate of whole eggs or isolated blastomeres may vary as much as 20 per cent, as indicated in Tables I and II. While it is difficult to distinguish errors of technique from biological variability, the results of duplicate tests suggest that this is mainly due to the latter. Variations of oxygen uptake of considerable magnitude have been observed, for example, in sea urchin eggs (Krahl, 1950).

In the experiments on the respiration of polar lobes, it was suspected that pretreatment with $MgCl_2$ may have caused a decrease in rate of oxygen uptake. This cannot be tested directly since there is no other known method for obtaining polar lobe cytoplasm in sufficient quantity for respiration measurements. However, as an indirect test, a series of experiments were made on whole eggs which had been exposed to $MgCl_2$ solutions much in the same way as for removing the polar lobes. The average oxygen uptake of the treated eggs was not significantly different from the controls. There still remains the rather unlikely possibility that $MgCl_2$ has a differential effect on the respiration of polar lobes as compared to the remaining cytoplasm; however, at present there is no method for conveniently testing this. The fact that the CD cells, which contain the polar lobe cytoplasm, show a low oxygen uptake would tend to confirm the conclusion that the low respiration of the polar lobe cytoplasm is not artificially induced. This also indicates that the lower respiration of the polar lobes is not due to the absence of nuclear material.

In Table II the oxygen uptake of polar lobes was compared with that of whole eggs. The latter, however, contain the lower respiring polar lobe material and for a comparison between the polar lobe cytoplasm and the remaining cytoplasm, one should include a correction for this. If one adjusts for the amount of polar lobe material actually pinched off (which has a volume of about 11 per cent of that of whole eggs) the correction is negligible. If it is assumed that the volume differences between AB and CD cells are due to polar lobe material, and furthermore that all polar lobe cytoplasm respires at the same low rate, then the correction becomes considerable. Such a calculation indicates that polar lobe cytoplasm has an oxygen consumption per unit volume 35 per cent lower than that of the remaining cytoplasm.

The assumptions made in the above calculation would be difficult to check; however, another calculation lends some support to the above interpretation. The respiration of the polar lobes cannot be compared directly with the AB cytoplasm, since by the time the AB cells can be counted and loaded in the diver, they have continued development and their rate of oxygen uptake increased accordingly. It is possible, however, to extrapolate the oxygen consumption curves of AB embryos (such as in Fig. 2) back to the time of completion of the first cleavage. If these values are expressed in terms of unit amount of cytoplasm and compared with the polar lobes (from Table II), the polar lobes are found to have an oxygen consumption 44 per cent lower than that of AB cytoplasm. While the above calculations are very approximate, they do, however, indicate that the value of 25 per cent obtained from the data of Table II is too low, and that there is probably a greater differential in rate of oxygen consumption between the polar lobe and the remaining cytoplasm than appears on first inspection.

Since the volume of an isolated polar lobe is about one fifth that of a CD cell, the lower respiratory rate of the isolated polar lobe cannot by itself account for the difference in respiratory rates of AB and CD cells. There is reason to believe, however, that not all the polar lobe cytoplasm is isolated by the $MgCl_2$ treatment.

Visual inspection of normally cleaving eggs indicates that the total polar lobe material is appreciably greater than the amount removed. If it is assumed that this remaining material has an equally low respiration as the isolates, then the difference between AB and CD cells can be reasonably accounted for.

Gradients of various types have been suggested as being important factors in embryonic development. The nature of these has not always been made clear; however, several investigators have suggested that they are physiological, or more specifically, metabolic in nature. Child (1941), for example, has proposed on the basis of differential reduction of dyes, a metabolic gradient along the polar axis as a controlling factor in development, particularly of the sea urchin. This has been suggested and often been interpreted as a gradient of oxidative activity. Considerable controversy has existed with regard to this interpretation, and at present there is a tendency to question it, especially in view of the fact that Holter and Lindahl (1940) found no difference in oxygen uptake of animal and vegetal halves of sea urchin embryos.

Although the present data show a regional difference in oxidative metabolism of Mytilus eggs, it cannot be concluded that this difference exists in the form of a gradient. Also, whether this difference is directly coupled with the differentiation of these areas is a point which must await further investigation.

The results of Navez and Harvey (1935), Shapiro (1935), and Ballentine (1940) indicate that oxidative enzymes are attached to submicroscopic granules which can be shifted by centrifugation. The polar lobe cytoplasm is relatively free of granules of the larger visible size and may be lacking to some extent in the smaller ones with attached oxidative enzymes. While there is no proof for this, it is a possible explanation for the lower oxidative rate of the polar lobe cytoplasm.

Ries (1939) has shown by cytochemical methods that specific regions of mosaic eggs are particularly abundant in oxidative enzymes. A striking example of localization of oxidases occurs in the myoplasm of the ascidian egg and one might suppose that this area would have a higher oxidative metabolism. Holter and Zeuthen (1944), however, found no difference between the oxygen uptake of the anterior and posterior blastomeres of the four-cell stages of Ciona.

The present work is the first indication that regions of a mosaic egg may be characterized by different respiratory intensities; however, it might be pointed out that the results are not particularly in accordance with those of cytochemical studies. Pitotti (1947) has suggested that oxidative enzymes tend to be correlated with cytoplasmic areas, the developmental fates of which are to form organs of movement. Thus, the ectoplasm of Berŏe eggs, the myoplasm of the ascidian egg, and the polar lobe of Myzostoma are positive with cytochemical tests for oxidative enzymes. While it is not known if the polar lobes of Mytilus exhibit a localization of oxidative enzymes as revealed by cytochemical tests, it is of interest that the respiratory intensity of the polar lobe is lower rather than higher than that of the remaining cytoplasm.

Summary

1. The oxygen consumption of isolated blastomeres and isolated polar lobes of the eggs of *Mytilus edulis* was measured by means of the Cartesian diver microrespirometer. The average oxygen uptake of CD embryos is $0.78 \pm .027$ μl. O_2/hr./mm.^{3} CD cytoplasm. This is 13 per cent lower than the average oxygen

uptake of AB embryos (0.90 ± 0.032) over a comparable period of development.

2. This lower respiratory rate of CD cytoplasm is undoubtedly due to the presence of the polar lobe cytoplasm. Isolated polar lobes have an oxygen uptake of $0.42 \pm .025$ μl. O_2/hr./mm.^{3} cytoplasm, which is 25 per cent lower than the average oxygen uptake of whole eggs during the time of polar lobe formation ($0.56 \pm .013$). Calculations by several methods suggest that the difference in respiratory rate between the polar lobe and the remaining egg cytoplasm may be appreciably greater than the above figure.

3. The rate of oxygen consumption of polar lobes remains constant after isolation from whole eggs whereas those of AB and CD embryos, as well as whole embryos, exhibit a gradual rise during subsequent development. Prior to hatching, whole embryos show a temporary decrease in oxygen consumption. This has been found to be due to a decrease in oxygen uptake of derivatives of the AB cell. Isolated CD embryos exhibit a steady rise in respiratory intensity during this period. This difference in rate of oxygen consumption of AB and CD embryos prior to hatching can, to some extent, be correlated with their rate of cell division.

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THE EFFECTS OF CERTAIN ANTITHYROID DRUGS ON THE UPTAKE OF RADIOACTIVE IODINE BY THE FROG THYROID[1]

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Within recent years a number of chemical substances which inhibit the activity of the thyroid gland have been extensively studied by Astwood (1943), MacKenzie and MacKenzie (1943), Astwood, Bissell and Hughes (1945), McGinty and Bywater (1945), VanderLaan and Bissell (1946a). In general, these antithyroid substances fall into three classes: (1) thiourea and its derivatives, (2) aniline derivatives, including the sulfonamides and other aminobenzene compounds, (3) thiocyanates.

One method of approach to the problem is the use of radioactive iodine (I^{131}) as a tracer for following the course of injected iodine in animals treated with thyroid-inhibitors. With radioactive iodine tracer techniques, it has been demonstrated by Franklin, Lerner and Chaikoff (1944) and Rawson, Tannheimer and Peacock (1944) that the oral administration of thiouracil to rats and chicks for periods of several days results in a marked reduction in the natural capacity of thyroid tissue to concentrate iodine. In another study, Larson, Keating, Peacock and Rawson (1945) demonstrated that a single injection of 10 mg. of thiouracil markedly inhibited the collection of radioactive iodine by the chick thyroid.

Such studies, for the most part, have dealt with warm-blooded animals. The present paper is a report of experiments carried out on the frog to determine (1) whether frog thyroids treated with thyroid-inhibitors concentrate iodine as effectively as those of control animals; (2) whether there are differences in the action of the three groups of antithyroid compounds; (3) whether there are any significant differences between warm-blooded and cold-blooded forms with respect to the mechanisms involved in the goitrogenic effects of these substances.

Materials and Methods

Male and female frogs (*Rana pipiens, Rana clamitans* and *Rana palustris*) of 50 to 65 grams body weight were kept in a large screened cage under dripping water in an air-conditioned room at 5° C.

[1] This paper is based on the author's dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the Catholic University of America.

I am indebted to Dr. W. G. Lynn for suggesting this problem and for many helpful suggestions during the course of the work. I am also grateful to the Reverend H. E. Wachowski and Dr. J. A. O'Brien for advice and guidance during the study and in preparation of the manuscript. Special thanks are due to Dr. F. Friedberg of the Department of Bio-chemistry of the Medical School of Howard University for generously permitting the use of apparatus for making the determination of radioactivity and for giving freely of his time to train the writer in the use of this apparatus. I am indebted to Dr. H. Branson of the Department of Physics of Howard University for allotting a supply of radioactive iodine for use in the experiment. The radioactive iodine was supplied through a grant to Howard University by the United States Navy Department.

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In the first series of experiments, groups of experimental animals were given single sub-cutaneous injections of the drugs to be tested. Those receiving thiourea or sulfanilamide were given 0.5 cc. of a 10 per cent solution; those receiving potassium thiocyanate were given 1 cc. of a 0.2 per cent solution. Twenty-four hours after the drug injection a dose of approximately 22.5 microcuries of carrier-free radioactive iodine in a sodium bisulphite solution was injected intraperitoneally into both experimental and control animals. Uniform groups of animals were sacrificed 20 minutes, 40 minutes, one hour, three hours, 8 hours, 24 hours, and 48 hours after injection of the radioactive iodine. The thyroids were removed, rinsed in frog Ringer's solution, blotted, weighed, pressed flat on clean microscope slides, and radioactivity counts made.

In the second series of experiments the experimental animals were given the same doses of the test compounds as above. At intervals of one hour, three hours, 8 hours, 24 hours, 48 hours, and, in some cases, 72 hours after injection of the test compounds, groups of animals were injected intraperitoneally with 22.5 microcuries of carrier-free radioactive iodine in a solution of sodium bisulphite. The control animals received only the radioactive iodine. The animals were sacrificed three hours after the injection of labeled iodine. The thyroids were removed and prepared for reading as above.

The first and second series of experiments showed that potassium thiocyanate disappeared from the circulation approximately 24 hours after its injection into the animal. For this drug a third series of experiments was performed in order to see if the curve for its effectiveness at successive time intervals was similar to the curves for thiourea and sulfanilamide. In this series of experiments the drug was injected and at one minute, 20 minute, 40 minute, one hour, three hour, 8 hour, and 12 hour intervals, the radioactive iodine was injected into experimentals and controls; all animals were killed three hours after injection of the iodine.

In the three series of experiments the collection of radioactive iodine by the thyroid glands was detected by serial counting at two-minute intervals with a Geiger-Muller counter. In each group of animals the count of radioactive iodine collected by the thyroids of the experimental animals was compared with that collected by the thyroids of the untreated controls and expressed as per cent of the control uptake of radioactive iodine. The count of radioactive iodine collected by the thyroids of the experimental groups divided by that of the control animals determines this per cent of control uptake.

Results

The thyroid glands of the control animals were normally pale and to the naked eye did not appear enlarged. The weights of these glands varied between 0.0008 and 0.0014 gm. and averaged 0.001 gm. Animals treated with thiourea had thyroid glands which were dark red, obviously vascular and enlarged. The weights of the thyroid glands of these animals varied between 0.0014 and 0.0023 gm. and averaged 0.0019 gm. Animals treated with sulfanilamide had thyroid glands which were dark red, vascular and much enlarged. The weights of this group of thyroids averaged 0.0015 gm. and varied between 0.0012 and 0.0018 gm. The thyroid glands of the potassium thiocyanate-treated animals were dark red, quite vascular and enlarged. The weights of the thyroid glands of these animals averaged 0.0012 gm. and varied between 0.0010 and 0.0014 gm. The injection of the drugs into the experimental

animals produced thyroid glands larger than those of the control animals in every instance. The increase in volume of the glands was apparent to the naked eye and seems to have been greater than was the increase in weight.

Effects of thiourea and sulfanilamide

The effect of a single injection of 0.5 cc. of a 10 per cent solution of thiourea upon the uptake of radioactive iodine by the frog thyroid is shown graphically in Figure 1. It will be seen that the curve for the experimental animals follows that

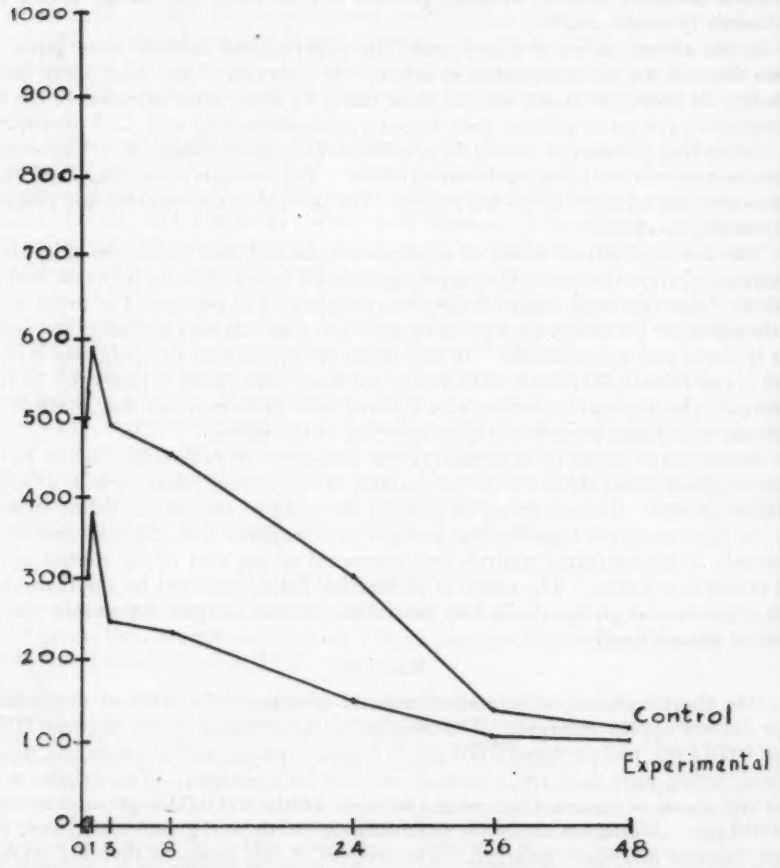

FIGURE 1. The effectiveness of thiourea as an inhibitor of the uptake of I^{131} by the frog thyroid at successive time intervals after injection of the drug. Each point on the graph represents the average of two separate series of experiments. The ordinate shows radioactivity counts per two minutes and the abscissa indicates the time intervals in hours after injection of radioactive iodine.

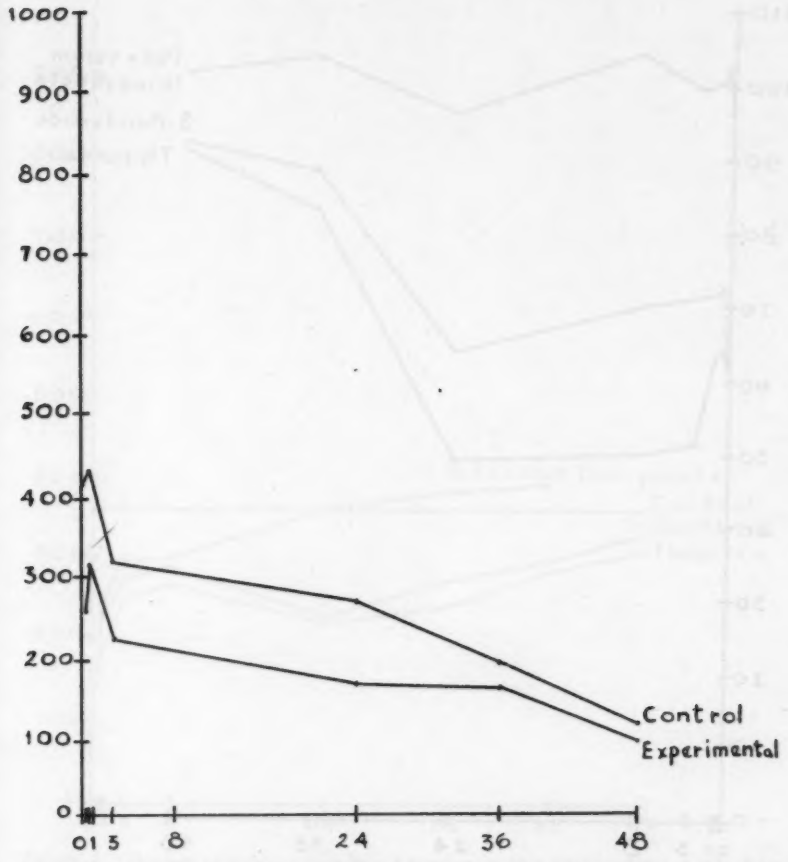

FIGURE 2. The effectiveness of sulfanilamide as an inhibitor of the uptake of I^{131} by the frog thyroid at successive time intervals after injection of the drug. Each point on the graph represents the average of two separate series of experiments. The ordinate shows radioactivity counts per two-minute intervals, and the abscissa indicates the time intervals in hours after injection of radioactive iodine.

for the controls but is consistently lower. The thiourea-treated animals show an uptake which is 62.0 per cent of that of the controls 20 minutes after the injection of radioactive iodine (24 hours and 20 minutes after administration of thiourea). The percentage falls steadily to a level of 48.5 per cent at the 24 hour period (48 hours after thiourea administration). Then, as would be expected, a gradual recovery is exhibited so that by 72 hours after thiourea administration the iodine uptake of the treated thyroids is approaching that of the controls. It is clearly indicated, therefore, that thiourea does affect the thyroid's ability to take up iodine and that,

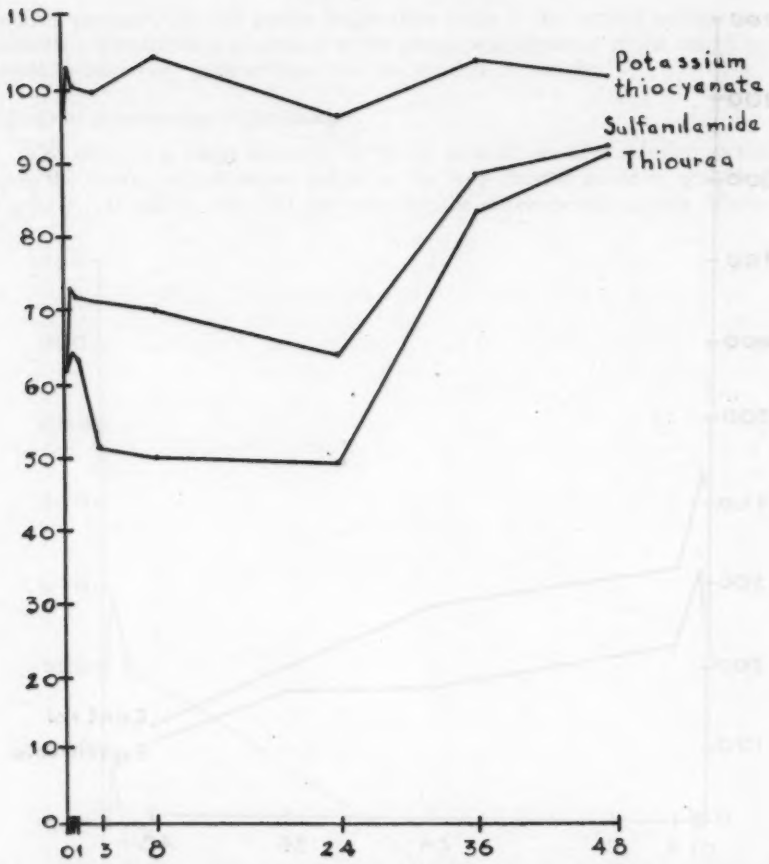

FIGURE 3. The per cent of control uptake of I^{131} by the frog thyroid is compared for thiourea-, sulfanilamide- and potassium thiocyanate-treated animals. Each point on the graph represents the average of two separate series of experiments. The ordinate shows the per cent of the control uptake of I^{131}, and the abscissa indicates the time interval in hours after its injection.

in the frog, a single dose of 0.5 cc. of a 10 per cent solution of thiourea has a maximum effectiveness about 48 hours after the drug is given.

The data for sulfanilamide are graphically presented in Figure 2. The results are clearly similar to those obtained by thiourea treatment, but the effect (at this dosage level) is somewhat less marked, the maximum reduction being only to 63.7 per cent of that of the controls. Another basis for comparison is given in Figure 3 where the curves are based on percentages of control uptake.

After the experiment in which the radioactive iodine was given 24 hours after administration of the thyroid-inhibiting drug and its uptake followed over a 48-hour

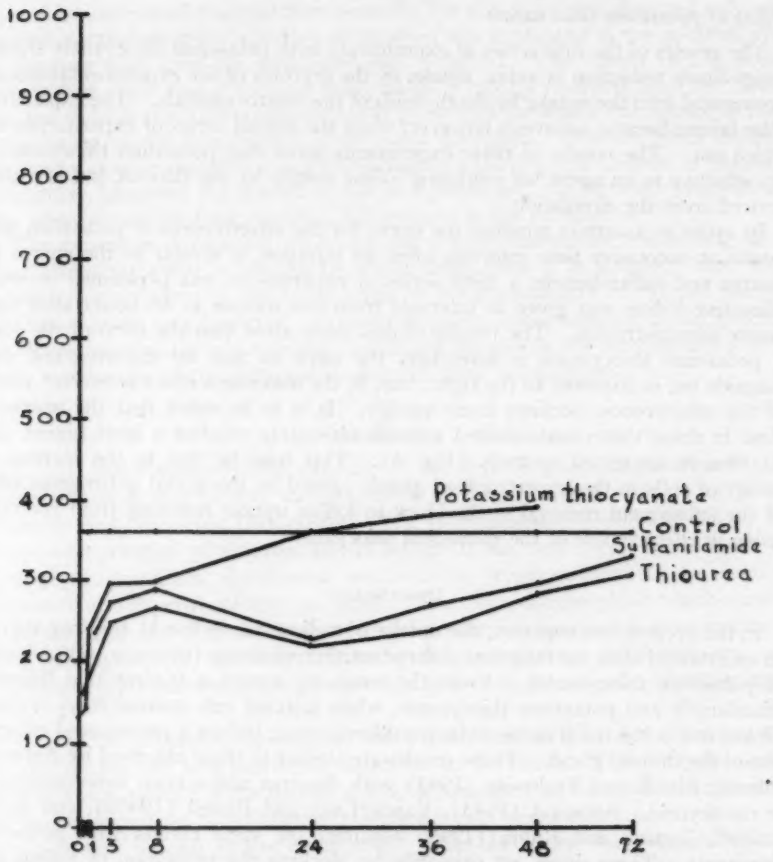

FIGURE 4. The rate of radioactive iodine absorption by frog thyroids treated with thiourea, sulfanilamide and potassium thiocyanate. Each point on the graph represents the average of two separate series of experiments for each drug. The ordinate shows the radioactivity counts per two-minute interval, and the abscissa indicates the time intervals in hours after injection of radioactive iodine.

period, a series of experiments was undertaken in which the radioactive iodine was given at varying times after the drug. The data for thiourea show that the drug effectively blocks iodine absorption almost immediately after its injection. In fact, the maximum effectiveness is seen in the animals which were injected with radioactive iodine only one hour after thiourea injection. With longer intervals the effect is gradually reduced although it is still perceptible after three days. For sulfanilamide the results are similar although again the degree of effectiveness with this dosage is somewhat less than that shown by thiourea.

Effects of potassium thiocyanate

The results of the first series of experiments with potassium thiocyanate showed no significant reduction in iodine uptake by the thyroids of the experimental animals as compared with the uptake by the thyroids of the control animals. The explanation of the failure became apparent, however, when the second series of experiments was carried out. The results of these experiments show that potassium thiocyanate is very effective as an agent for inhibiting iodine uptake by the thyroid, but is rapidly removed from the circulation.

In order to ascertain whether the curve for the effectiveness of potassium thiocyanate at successive time intervals after its injection is similar to the curves for thiourea and sulfanilamide, a third series of experiments was performed in which radioactive iodine was given at intervals from one minute to 48 hours after thiocyanate administration. The results of this study show that the form of the curve for potassium thiocyanate is essentially the same as that for thiourea and sulfanilamide but is displaced to the right; that is, the maximum effect is exerted earlier and the effectiveness declines more quickly. It is to be noted that the uptake of iodine in these thiocyanate-treated animals ultimately reached a level higher than that seen in untreated controls (Fig. 4). This may be due to the increase in number of cells in the hypertrophied glands caused by the initial goitrogenic effect and the subsequent removal of the block to iodine uptake resulting from the rapid decline in effectiveness of the potassium thiocyanate.

Discussion

In the present investigation, the uptake of radioactive iodine by the frog thyroid was ascertained after the injection of three antithyroid drugs (thiourea, sulfanilamide and potassium thiocyanate). From the foregoing results it is clear that thiourea, sulfanilamide and potassium thiocyanate, when injected into normal frogs in doses that are not in the usual sense to be considered toxic, induce a pronounced enlargement of the thyroid gland. These results are similar to those observed by Astwood, Sullivan, Bissell and Tyslowitz (1943) with thiourea and certain sulfonamides on the rat thyroid. Astwood (1943), VanderLaan and Bissell (1946b), and Wolff, Chaikoff, Taurog and Rubin (1946) obtained the same results with potassium thiocyanate. These drugs act primarily by blocking the utilization of iodine, and thyroid hyperplasia and hyperemia occur as secondary compensatory responses to the increased thyrotropic hormone output by the pituitary. The basic system governing the structure and function of the thyroid gland appears to be an interrelation and balance between the thyrotropic activity of the anterior pituitary and the production of a calorigenic substance from the thyroid gland.

By the use of the radioactive iodine technique, it was found that thiourea and sulfanilamide still exert a pronounced inhibitory effect on the thyroid twenty-four hours after injection, but this prolonged effect was not obtained with potassium thiocyanate. Collection of radioactive iodine by thyroids of potassium thiocyanate-treated frogs is similar to that observed for the rat thyroid by Franklin, Chaikoff and Lerner (1944). They observed that thyroid slices *in vitro* fail to absorb radioactive iodine in the presence of potassium thiocyanate. However, the thyroids of some potassium thiocyanate-treated frogs concentrated a greater per cent of the

injected labeled iodine than did their controls, but this effect was observed only when the time interval after the injection of the drug was prolonged to the point at which its disappearance from the circulation had occurred. The results of the present investigation show that potassium thiocyanate is rapidly removed from the circulation, but so long as a high concentration is present in the blood, the uptake of radioactive iodine by the frog thyroid is definitely depressed. The inhibitory action of this drug was observed after a single injection of a 0.2 per cent solution of potassium thiocyanate, provided the labeled iodine is injected and the thyroids removed at a time when potassium thiocyanate is still present in the circulation.

The experiments reported above do not permit conclusions concerning all the details bringing about inhibition by these drugs, but it is assumed that iodine absorption is only one of the many steps in the manufacture of thyroid hormone which is in some way hindered. A more complete understanding of the processes controlling normal thyroid function would aid greatly in the interpretation of the whole mechanism of the goitrogenic effect of these compounds.

Summary

1. The iodine-concentration capacity of the thyroid gland, as measured by the uptake of radioactive iodine, was depressed in frogs injected with thiourea, sulfanilamide and potassium thiocyanate. The subcutaneous injection of 0.5 cc. of a 10 per cent solution of thiourea and of sulfanilamide, and 1 cc. of a 0.2 per cent solution of potassium thiocyanate into normal frogs resulted in an enlarged, hyperemic and hyperplastic thyroid gland. The thyroid hyperplasia which occurred under the influence of these drugs was a secondary compensatory response to the increased thyrotropic hormone output by the hypophysis. Inhibition was most pronounced with thiourea, sulfanilamide and potassium thiocyanate following in that order.

2. Injected potassium thiocyanate was rapidly removed from the blood stream, and its elimination was practically complete in 24 hours. However, the iodine-concentrating capacity of the thyroid gland, as measured by the uptake of radioactive iodine, was depressed by potassium thiocyanate, provided a high concentration of the drug was still present in the circulation. After 24 hours, the enlarged thyroids of the potassium thiocyanate-treated frogs had a greater capacity than normal for absorbing injected radioactive iodine.

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STUDIES OF THE DAILY RHYTHMICITY OF THE FIDDLER CRAB, UCA. MODIFICATIONS BY PHOTOPERIOD[1]

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Since the first report of a persistent diurnal chromatophore rhythm in Uca by Abramowitz (1937), considerable work has been done in this laboratory as well as others in an attempt to elucidate the mechanisms controlling this periodic response. These studies, together with the reports of workers concerned with other manifestations of diurnal periodicity of function, have produced a literature which is extensively reviewed in Welsh (1938) and Webb (1950).

Light is the most obviously diurnally periodic feature of most environments and for this reason has been a popular experimental variable in studies of the control of diurnal rhythms. However, despite this fact, no work has been reported studying the effect of varying the extent of the daily photoperiod on the quantitative relations of a diurnally periodic response. The diurnal chromatophore rhythm of Uca can be readily observed and use of the Hogben-Slome scale for measuring chromatophore dispersion allows an accurate quantitative description of the characteristics of this rhythm. These characteristics make Uca a suitable experimental animal for such purposes. Thus the experiments to be reported were designed to contribute to an understanding of the mechanisms controlling diurnal periodicity by studying the character of the responses of this chromatophore rhythm to variations in the length of the daily light period to which the animals are exposed.

Material and Methods

All animals used in these experiments were male specimens of *Uca pugnax* collected in the vicinity of Woods Hole, Massachusetts. The animals were placed in white enameled pans in groups of twenty and subjected either to illumination at an intensity of 100 foot candles or to darkness, depending on the protocol which the group concerned was to follow. This was done not later than two hours after collection. The time of collection was between 10 A.M. and 4 P.M., this time being chosen to avoid the dark period of as many of the experimental groups as possible in order to provide constant experimental treatment.

Two sets of experiments are to be reported which differ to some extent in protocol. All succeeding references to light refer to light at an intensity of 100 foot candles furnished by the laboratory fluorescent lights. Groups were subjected to indicated periods of darkness by placing them in an adjacent darkroom. In both sets of experiments the condition of the black chromatophores in each experimental group was determined at 1, 4, 7, and 10 A.M., and 1, 4, 7, and 10 P.M. by

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recording the extent of dispersion for each animal in terms of the Hogben-Slome scale.

The first set of experiments was begun on July 26, 1950 and terminated on August 9, 1950. The following groups were maintained and the black chromatophore activity of each ascertained in the manner described above.

1) A control group maintained in constant darkness from 4 P.M. July 26 until the experiments were concluded.
2) A group exposed to light from 7 A.M. to 1 A.M. and darkness from 1 A.M. to 7 A.M. daily (18-hour photoperiod).
3) Exposed to light from 1 A.M. to 7 P.M. (18-hour photoperiod).
4) Exposed to light from 4 A.M. to 10 P.M. (18-hour photoperiod).
5) Exposed to light from 7 A.M. to 1 P.M. (6-hour photoperiod).
6) Exposed to light from 1 P.M. to 7 P.M. (6-hour photoperiod).
7) Exposed to light from 10 A.M. to 4 P.M. (6-hour photoperiod).

In each case not specified above, the animals concerned spent the remainder of each 24 hour period in darkness and were accorded the same treatment daily for the duration of this portion of the experiments. On July 31 groups 2 through 7 above were placed in darkness after exposure to light as outlined above and so maintained until the experiments were terminated on August 9. There are two exceptions to this statement; one-half the animals remaining in groups 3 and 4 were placed in darkness at 1 P.M. and 4 P.M., respectively, on July 31. It was found that this treatment altered neither the amplitude of the rhythm nor the magnitude of the dispersed phase and produced only a transient effect on the time of concentration. After this had been ascertained, these animals were discarded and are not included in the calculation of the magnitude of the dispersed phase for the groups which will be discussed subsequently. Thus, at the end of this set of experiments, groups 3 and 4 were smaller than the others, containing respectively seven and five animals. In no other case did less than 10 of the original 20 animals in each group survive the experimental period.

The second set of experiments was begun on August 14, 1950 and terminated on August 24, 1950. The following groups were maintained and black chromatophore activity ascertained in each case as before.

1) A control group maintained in constant darkness as before for the duration of the experiments.
2) A second control group was maintained in conditions as closely approximate to normal with respect to light periodicity as feasible. The animals were exposed to daylight in a wooden collecting bucket. No attempt was made to control or measure intensity but the animals were shielded from direct sunlight to avoid lethal temperatures. Sea water in the bucket was renewed daily and the animals were shielded from rain when necessary.
3) Exposed to light from 4 A.M. to 10 P.M. daily (18-hour photoperiod).
4) Exposed to light from 10 A.M. to 4 P.M. daily (6-hour photoperiod).

On August 18, groups 3 and 4 were placed in darkness after their respective exposures to light (in the case of group 2 at 1 A.M. on August 20) and so maintained until the experiments were terminated. In no case did less than 9 of the original 20 animals in each group survive the experimental period.

The diurnal rhythm of the black chromatophores persisted in all cases. For the purposes of analysis of the data this rhythm has been treated as a daily excursion into the dispersed phase and direct measurements of the course of each 24 hour cycle transformed into a parameter which is proportional to the area swept out by the dispersed phase. The formula used for this calculation is

$$A = \frac{\sum_{i=1}^{n} (x_i - 1)}{8}$$

"i" refers to the time of the reading concerned. "i = 1" was arbitrarily taken as the initial point of minimum dispersion in the cycle under consideration.

"x_i" is the average extent of dispersion of the black chromatophores of the group concerned as measured in terms of the Hogben-Slome scale at the time "i".

"n" is the number of the reading which indicates the second point of minimum dispersion and the conclusion of the cycle considered. The magnitude of "n" varied between 8 and 10. Reading "n" for one cycle is then reading "1" for the cycle immediately succeeding.

This formula essentially gives an expression for the average dispersion of the black chromatophores in the course of one cycle, on the assumption that each cycle occurs in 24 hours. This assumption is thoroughly justified on the basis of the observational data.

Results

The values for the average dispersion of the black chromatophores for each group calculated, using the formula explained above, are arranged in tabular form

Table I

Daily magnitude of the dispersed phase in each group of the first series of experiments. All groups in constant darkness after the fifth day

Group..... Day	1	2	3	4	5	6	7
1	2.10	2.33	2.45	2.39	1.63	1.81	1.94
2	2.36	2.45	2.66	2.66	1.76	2.00	2.11
3	2.71	2.46	2.65	2.64	2.04	1.81	2.09
4	2.50	2.64	2.61	2.64	2.16	2.07	2.21
5	2.32	2.69	2.76	2.81	2.11	1.89	2.14
6	2.56	2.57	2.23	2.56	2.26	1.99	2.11
7	2.53	2.23	2.29	2.28	2.16	1.93	2.13
8	2.51	2.25	2.29	2.35	2.28	2.36	2.09
9	2.89	2.49	2.32	2.50	2.16	1.99	2.14
10	2.61	2.45	2.41	2.06	2.20	2.23	2.10
11	2.46	2.29	2.20	2.05	1.97	1.76	1.98
12	2.53	2.39	2.21	2.18	2.18	2.02	1.96
13	2.58	2.50	2.39	2.13	2.02	2.06	1.85
Av. (6–13)	2.58	2.40	2.30	2.26	2.16	2.04	2.04
SD	±.08	.11	.06	.16	.08	.13	.09
Aggregate average		2.32 ± .14			2.08 ± .11		

in Tables I and II. Inspection of the values for the first five days of the first series of experiments as presented in Table I is sufficient to validate the statement that for this period the responses of groups 2, 3, and 4 are similar and differ significantly from the responses of groups 5, 6, and 7. Further, the responses of groups 5, 6, and 7 are similar. The antepenultimate row of Table I lists the average value of the magnitude of the dispersed phase in cycles 6 through 13 of the groups concerned and the penultimate row gives the standard deviation for this value. These cycles were chosen as representative of what may be called the resting level of the rhythm in constant darkness. It is seen that these average values fall naturally into three sets comprised of group 1, groups 2, 3, 4, and groups 5, 6, 7. The last row of Table I lists the averages of cycles 6 through 13 for groups 2, 3, 4, and groups 5, 6, 7 considered as aggregates, together with the standard deviation of these values. This constitutes evidence for the conclusion that the magnitude of the dispersed phase in the rhythm, both during the periodic light exposure and in constant dark-

TABLE II

Daily magnitude of the dispersed phase in the second series of experiments. All groups in constant darkness after the fifth day except 2 which was placed in darkness on the sixth day

Group..... Day	1	2	3	4
1	1.35	2.30	2.23	1.59
2	1.38	2.11	2.44	1.79
3	1.59	2.01	2.54	1.69
4	1.80	2.13	2.48	1.80
5	1.95	2.06	2.65	1.84
6	2.18	2.20	2.19	1.84
7	2.11	1.54	2.33	1.94
8	2.13	1.41	2.32	1.79
9	2.18	1.59	2.16	1.73
10	1.99	1.80	2.09	1.67
Av. (6–10)	2.12		2.22	1.79
Av. (1–6)		2.13		
SD	±.05	.08	.09	.08

ness afterwards, is influenced by the length of daily exposure to light but is uninfluenced within the limits of the experimental procedure by the time of day at which the animals are exposed.

Table III lists the deviations of the minimum dispersion point at the start of each cycle for each group from an arbitrary zero point of 1 A.M. Two conclusions are supported by these tabulated data. On July 26, 1950, time of sunrise was 0530 Eastern Daylight Saving time and time of sunset was 2008. This places the middle of the naturally-occurring dark period at −0.18 in terms of the units used in Table III. Similar considerations place the middle of the naturally-occurring dark period on August 14, 1950 at −0.23. Given these facts, reference to the average values in the last row of Table III for series I, group 1 and series II, groups 1 and 2 allows the conclusion that the point of minimum dispersion does not normally coincide with the middle of the natural dark period nor does it bear any simple fixed relation to it. Evidence is provided for a second conclusion by inspection of the average deviations

for series I, groups 2 and 3. Both groups were exposed to light for 18 hours per day but the middle of the experimental dark period was at 4 A.M. and 10 P.M. respectively. The respective increments with respect to the middle of the normally-occurring dark period on July 26 are 3.18 and −2.82. The measured increments are 2.96 and −1.86. That the induced shifts are permanent is attested by the fact that the averages of points 6 through 14 (*i.e.*, after the animals were in constant darkness) are 2.67 and −2.33 in the two cases, manifesting no tendency to drift back to the control value represented by group 1 in this series. The value for series II, group 3 is further evidence for the existence of a persistent shift. It should be pointed out that since measurements were made at three-hour intervals, the error for each of the tabulated values is high, of the order of an hour and a half. However, the probable error of the average of a number of such values is considerably smaller and does not vitiate the conclusions drawn.

TABLE III

Daily deviations of the point of minimum dispersion from 1 A.M. as an arbitrary zero point. Time in hours

	Series I							Series II			
Group... Day	1	2	3	4	5	6	7	1	2	3	4
1	0	3.0	0	0	0	0	0	0	−3.0	1.5	−1.5
2	0	3.0	−1.5	0	1.5	1.5	1.5	1.5	0	0	−1.5
3	1.5	3.0	−1.5	1.5	3.0	3.0	3.0	−1.5	0	0	−1.5
4	0	3.0	−1.5	0	−1.5	0	0	0	−3.0	0	−1.5
5	3.0	3.0	−3.0	0	−1.5	−3.0	0	−1.5	−1.5	0	−1.5
6	0	3.0	−1.5	0	−1.5	0	−1.5	−1.5	−1.5	0	−3.0
7	3.0	3.0	−3.0	−3.0	−3.0	3.0	0	−1.5	−1.5	0	0
8	1.5	1.5	−3.0	−3.0	0	0	0	−3.0	−1.5	0	−1.5
9	0	1.5	−1.5	−3.0	0	0	0	−1.5	0	0	−1.5
10	1.5	3.0	−3.0	−1.5	0	0	0	−3.0	−1.5	0	−3.0
11	1.5	3.0	−3.0	−3.0	1.5	3.0	0				
12	0	3.0	−3.0	−1.5	−1.5	−3.0	−1.5				
13	0	3.0	−3.0	−1.5	0	0	0				
14	0	3.0	0	0	−1.5	0	0				
Av.	.86	2.78	−2.04	−1.07	−.32	.32	.11	−1.20	−1.35	.15	−1.65

Figure 1 displays graphically the variation in the magnitude of the periodic dispersion of the black pigment in the first series of experiments. It has been previously argued that the time of day at which the animals were exposed to light was immaterial in a consideration of induced shifts in this magnitude, so that the data from groups 2, 3, and 4 have been averaged to give the points representing the response of animals exposed to 18 hours of light daily and the data from groups 5, 6, and 7 contribute in the same way to the curve for animals exposed to 6 hours of light daily. Figure 2 presents the same information for groups accorded similar treatment in the second series of experiments and in addition records the variations in dispersion for the additional control group maintained under normal light conditions.

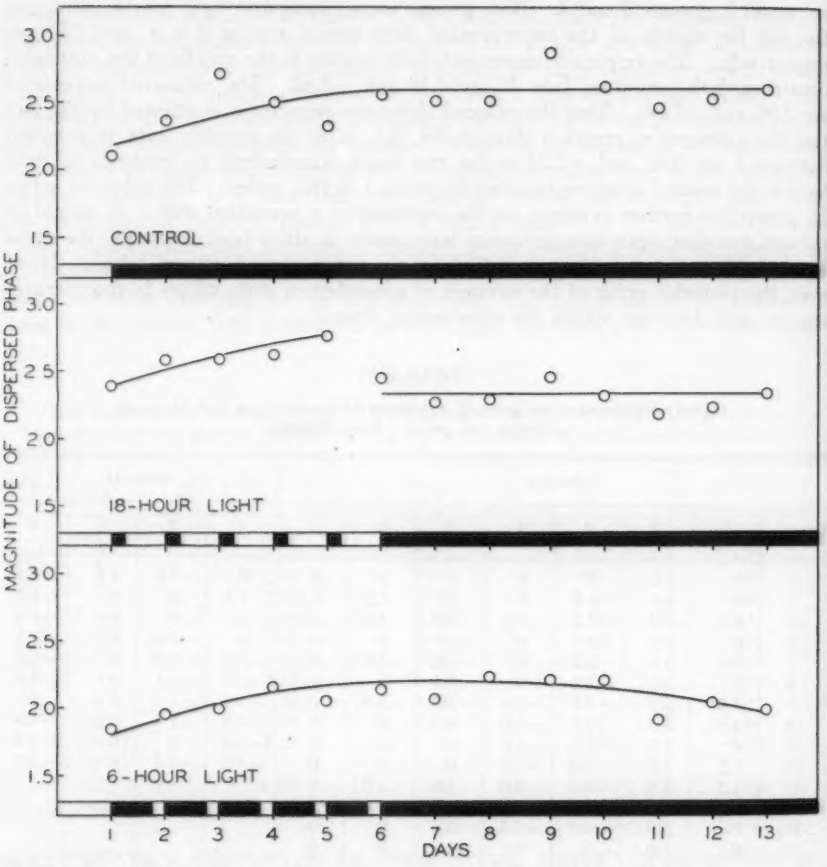

FIGURE 1. The responses of the magnitude of the dispersed phase of the chromatophore rhythm in the three groups of the first series of experiments. The white and black blocks under each curve are a diagrammatic representation of the relative lengths of each 24 hour period spent in light and darkness respectively.

There are several features of these graphs which are of interest. It will perhaps be simplest to discuss the responses of the groups receiving similar treatment in the two sets of experiments.

One of the most striking features of the control values in both figures is their smooth increase over a period of several days from an initial relatively low magnitude to attain finally a resting level. This is particularly pronounced in series II (Fig. 2) where the effect manifested by the controls in darkness is repeated almost quantitatively by the controls maintained in normal daylight when placed in constant darkness after the sixth day. This portion of the response is less evident in series I

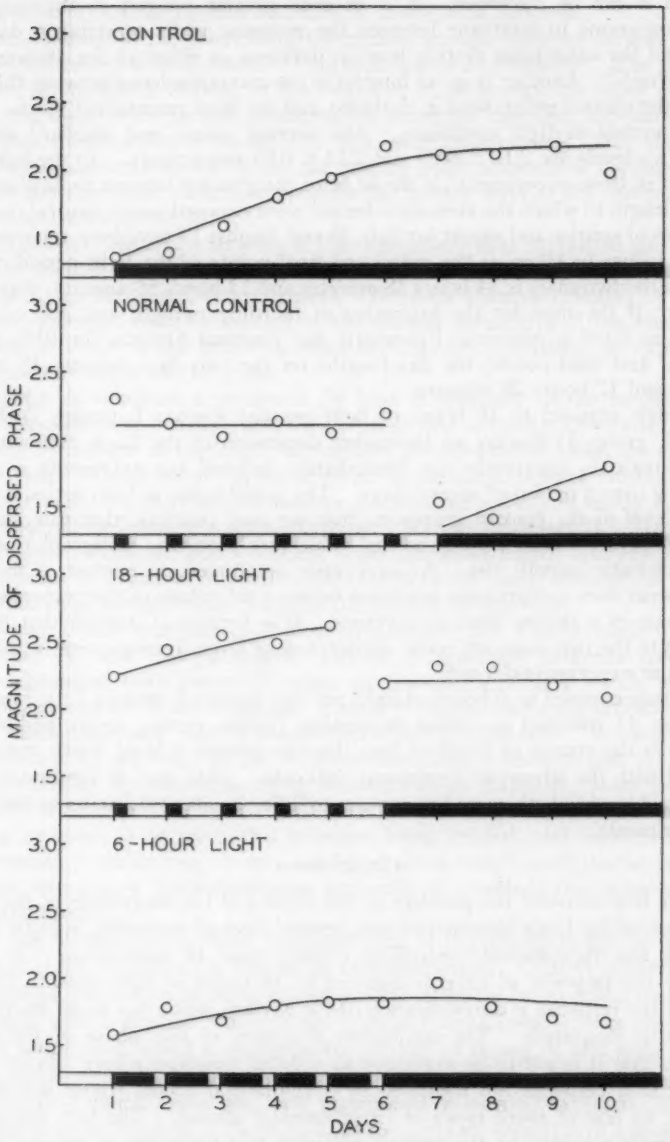

FIGURE 2. The responses of the magnitude of the dispersed phase of the chromatophore rhythm in the four groups of the second series of experiments. The white and black blocks beneath each curve have the same significance as in Figure 1.

although it can be discerned. It is of even greater interest in that no similar depression seems to intervene between the response while receiving a daily light ration and the subsequent resting level in darkness in either of the 18-hour or the 6-hour groups. Another point of interest is the correspondence between the resting level of the control group kept in darkness and the level maintained by the controls kept in normal daylight conditions. The average values and standard deviations for the two levels are 2.12 ± 0.05 and 2.13 ± 0.08 respectively. In the light of the character of these experiments, it would be of the greatest interest to be able to state the day-length to which the animals collected were exposed under natural conditions. The times of sunrise and sunset for July 26 and August 14 have been reported above. If these values be taken as the initial and final points of the light period, the day-length in the two cases is 14 hours 38 minutes and 13 hours 58 minutes, respectively. However, if the time for the beginning of morning twilight and end of evening twilight, as listed in American Ephemerix and Nautical Almanac for 1950, be taken as initial and final points, the day-lengths on the two days become 18 hours 39 minutes and 17 hours 28 minutes.

Animals exposed to 18 hours of light per day (series I, groups 2, 3, 4, and series II, group 3) display an increasing dispersion of the black chromatophores. This increase is apparently not immediately induced but represents a response extending over a period of several days. The initial value in both series is near the resting level of the control groups so that we may conclude that this cumulative increase operates on an initially normal value (see preceding paragraph) producing a characteristic smooth rise. As previously mentioned, in neither of the sets of experiments does a depression intervene between this phase of the response and the assumption of a resting level in darkness. It is further of interest that the levels assumed in the two cases are quite similar despite large discrepancies in the control and 6-hour experimental levels.

Animals exposed to 6 hours of light per day (series I, groups 5, 6, 7 and series II, group 4) manifest an initial depression (below resting level) and then rise steadily in the course of three or four days to assume a level which remains unchanged with the advent of continuous darkness. This level is significantly below the control level in both cases but appears to differ by about 0.2 units in the two sets of experiments.

Discussion

If we first consider the problem of the control of the magnitude of the periodic dispersion of the black chromatophores, several lines of reasoning indicate that two more or less independent controlling centers must be postulated. Let us first examine the response of animals exposed to 18 hours of light daily. The initial phase of the response is a modification of the rhythm which has as its starting point the normal magnitude. The cumulative character of this phase of the response indicates that it is not to be explained as a direct response where "direct" is used to refer to a response not mediated by a rhythmical center whose activity is influenced by one or more types of environmental stimuli. The fact that the level subsequently assumed by the animals receiving this treatment in series I is significantly below the control level, although the values attained in the initial phase of the response are significantly above this level, indicates that the center whose

activity was cumulatively modified to produce the initial increment is not responsible for the level at which the rhythm stabilizes in continuous darkness.

An examination of the behavior of the control groups furnishes further support for the hypothesis of a dual control of chromatophore rhythmicity. The period of depression or inhibition evident in the response of all groups on being placed in constant darkness from normal conditions is smoothly released, indicating again a cumulative modification of the activity of a controlling center. That this is not the controlling center responsible for the resting level assumed in darkness is indicated by the significant difference between the resting level of the control groups in the two sets of experiments.

Let us consider the characteristics of the two centers thus supported and attempt to describe insofar as possible the stimuli by which the activity of each is modified and the relations between the two centers. We have then two centers, A and B, which are concerned with the control of the diurnal periodicity of the black chromatophores of Uca. If the following characteristics are postulated, an explanation of the reported results can be given.

1) Center A manifests a persistent 24 hour rhythmicity in the secretion of a black-dispersing principle and the extent of this secretory activity is modified only by changes in the length of the daily photoperiod to which the animal is exposed. In addition to a direct effect on the stage of black chromatophore dispersion, this secretion, or another concomitant activity of center A, exerts a controlling influence on the rhythmical activity of center B. Total darkness or a daily photoperiod below some threshold length (no experimental evidence is available to permit a choice between these alternatives) is not effective as a stimulus in changing the activity of center A.
2) Center B also manifests a 24 hour rhythmicity but a more labile one than center A, and less persistent. Center B gradually becomes completely synchronized with center A when environmental stimuli which normally influence center B are removed. Center B has as mediating secretions both a black-dispersing and a black-concentrating substance. It responds to darkness by secretion of the black-concentrating substance and to light by secretion of the black-dispersing substance at its disposal. More precisely, there is an intensity *i* such that at or below this intensity a black-concentrating principle is secreted, this response being graded, and there is another intensity *j*, which may or may not be identical with *i*, such that at or above this intensity a black-dispersing principle is secreted, this response also being graded.

With two centers having these characteristics, it is possible to give a coherent explanation of the results of the two series of experiments. Control groups, it will be remembered, responded to being placed in total darkness by an initial depression in magnitude with a gradual return to an original resting level. Since there were no stimuli which could effect a change in the activity of center A, and since centers A and B would normally be acting synergistically, it is reasonable that the resting level in darkness should equal the level maintained in normal daylight. Further, since center B would respond to darkness with an increased secretion of a concentrating principle, a depression of the rhythm magnitude is to be expected until A regains complete control of the periodic activity of B.

When animals are placed in 18 hours of light at an intensity of 100 foot candles the situation is somewhat complicated. Under normal conditions, the animal would be subjected to periods of low intensity at the beginning and at the end of the photoperiod, from which it follows that center B would be activated to produce a dispersing substance during only a portion of the normal day-length exposure. However, under experimental conditions the animals were exposed to an intensity of 100 foot candles throughout the photoperiod. Thus, B was activated to produce a dispersing substance at a time when it had not been so activated under natural conditions. Such activation modified not only the immediate response of B but the character of its rhythmical activity so that as the stimulus was repeated on successive days, the reaction of center B to light became more and more effective in producing chromatophore dispersion. Thus, we observed a cumulative increase in magnitude as the initial response of these groups. Although center B continued its increased activity in the production of dispersing substance after the animals were placed in continuous darkness, this in itself also operated as a stimulus to the production of a black-concentrating substance. Both of these effects would be transient as center A gradually established control of the activity of B, and since they are antagonistic, only one of them could be expected to be observed and it should be less pronounced than it would be if operating alone. In fact, neither trend is observable. On the basis of this hypothesis, since the natural photoperiod on July 26 was greater than 18 hours (18 hours 39 minutes), center A should stabilize the rhythm at a level somewhat below the control level. The natural day-length on August 14 being 17 hours 28 minutes, the resting level in 18-hour animals should exceed that for the controls. Both of these expectations are confirmed. It should be pointed out in this connection that there is no reason to expect the activity of center A to be linearly related to the length of the photoperiod, so that it suffices to show one effect is greater than another, provided the stimulating day-length for the first is longer without regard to quantitative correspondence of increments. Finally, it is reasonable that the resting level attained by the 18-hour animals in darkness should be the same despite discrepancies in control level if center A is responsible for the control of this response.

In the response of animals exposed to 6 hours of light daily, center B is responsible for the initial drop in magnitude by its secretion of concentrating substance in response to the increased darkness and this effect is later supported by the induced shift in activity of center A. However, since center A responds slowly to changes in the length of the light period, the total effect will be a slow rise mimicking that shown by control groups when placed in darkness. For the same reasons which were adduced in the discussion of the reaction of the 18-hour animals, either an increase or a decrease in magnitude could be produced when the animals were placed in continuous darkness and neither was observed (the decrease observed in both sets in the course of several days is probably too small to be significant). On the basis of this hypothesis it is reasonable that the levels in the two cases were not the same since, by hypothesis, center A responds slowly to a change in light period. This argument does not apply to the case of the 18-hour animals since the experimental value in that case was quite close in both sets of experiments to the natural photoperiod.

Centers A and B discussed in the preceding hypothesis are in no way incompatible with centers 1 and 2, respectively, of Brown and Webb (1949), which were postulated

to account for other responses of the rhythmic mechanism of Uca; in fact, they even exhibit the same intrinsic characteristics. They are, therefore, assumed to be the same and this work is considered to provide striking support for the earlier hypothesis and to describe further properties of these two postulated centers.

Figure 3 is a diagrammatic representation of the mechanism which has been hypothesized to account for the responses of the daily rhythmicity observed during these experiments. In conformity with the earlier work, centers A and B are here indicated as 1 and 2.

With regard to the elements involved in this hypothesis, there is no reason to be concerned with the identity or non-identity of the dispersing principles whose liberation is controlled by A and B. The hypothesis requires only that there be two controlling centers, each of which has at its disposal a dispersing substance. Although it is not necessary that these two centers be physically discrete, it is possible that they are. Thus, it is of interest that both the sinus gland and the central nervous system in Uca elaborate black-dispersing substances (Sandeen,

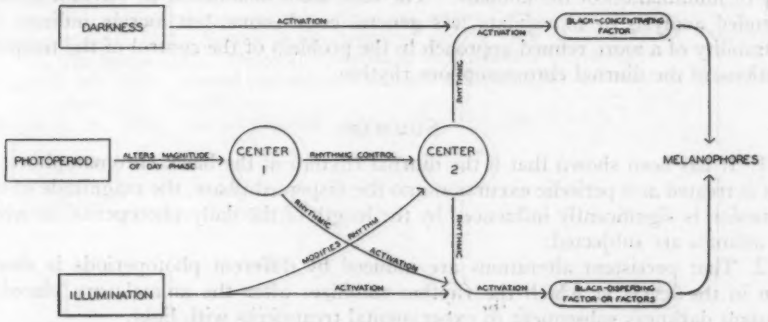

FIGURE 3. A diagrammatic representation of the postulated control mechanism of the black chromatophore rhythm of Uca.

1950). There is no direct evidence for the existence of a black-concentrating principle but other work done in this laboratory (Brown and Hines, in press) provides indirect support for its postulation.

The complexity of this controlling mechanism leads inevitably to speculation concerning the reasons for such elaborate systems. The idea has often recurred in the invertebrate endocrine literature that chromatophorotropins are simultaneously concerned with more basic functions. If, purely speculatively, this reasoning is followed, one may ask what functions would be thus controlled by the photoperiod. Panouse (1943, 1944, 1946), Brown and Jones (1949), and Stephens (1951) have demonstrated clearly the endocrine nature of the control of ovarian maturation, and the last investigator has shown a role of photoperiod in the decapod sex cycle. We have here a function which is presumably dependent on responses to change in day-length but which also requires that the responding system be uninfluenced by large short term variations in photoperiod. On the other hand, Edwards (1950) and others have pointed to the significance of endocrines in the diurnal activity rhythm

of the decapods. This, in contrast, requires an elastic response system capable of following short term variations. It is obvious that two such functions, if combined, produce a total chromatophorotropin system similar to the one described. Center A in our hypothesis represents an inelastic response mechanism whose characteristics are such as to make it suitable for the control of the sex cycle. Center B possesses the necessary lability for control of a function such as locomotor activity rhythm which must be warped to the exigencies of short-term environmental variations. Although there is some scattered evidence that some chromatophorotropins have other systemic effects in the decapods, such evidence can afford only general support for the possibility of this kind of interrelation which must remain speculation until further experimental evidence is accumulated. On the other hand, it is quite possible that these same two centers will be shown to exert their influences directly upon other nervous and endocrine mechanisms than those involved in color changes.

Table III provides evidence that permanent shifts in the relation of the phases of the diurnal rhythm to solar time, of the order of two to three hours in either direction from an arbitrary zero point, can be induced by suitable variation in the time of illumination of the animals. The data seem insufficient to warrant a more extended analysis or to validate less general conclusions, but merely indicate the desirability of a more refined approach to the problem of the control of the temporal relations of the diurnal chromatophore rhythm.

Summary

1. It has been shown that if the diurnal rhythm of the black chromatophores of Uca is treated as a periodic excursion into the dispersed phase, the magnitude of this excursion is significantly influenced by the length of the daily photoperiod to which the animals are subjected.

2. That persistent alterations are induced by different photoperiods is clearly seen in the levels at which the rhythm stabilizes after the animals are placed in constant darkness subsequent to experimental treatments with light.

3. The time of an 18-hour period of illumination between 1 A.M. and 1 A.M. appears not to influence the induced magnitude of the rhythm. The time of a 6-hour period of illumination between 7 A.M. and 7 P.M. is similarly immaterial. However, it has been shown that variations in the time of day within these limits of the period of illumination can produce corresponding and persistent shifts in the relation of the phases of the rhythm to solar time. These alterations may be in either direction from an arbitrary zero point.

4. In addition to the induction of persistent modifications of the rhythm by photoperiod, the character of the rhythm has been shown to be influenced also by illumination and by darkness. These latter effects are transient when the animals are no longer exposed to alternating periods of light and darkness.

5. An analysis of the data clearly indicates the operation of two centers of rhythmicity, such that the activity of the first is influenced by the length of the photoperiod to which the animal is exposed while the activity of the second is influenced by the duration and intensity of light to which the animal is exposed daily, by the duration of the daily dark period, and by the activity of the first center.

6. An adaptive significance for this duality of the control of the rhythm is suggested.

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THE UPTAKE OF VANADIUM BY TUNICATES[1]

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The ability of certain ascidians to concentrate vanadium was first reported in 1911 by Henze (1911), who found this element in the blood of four species. Since then more than a score of papers have extended these investigations.[2] The scope of the above work has included the analyses of various species of ascidians to determine the presence or absence of vanadium, and experiments to indicate any possible physiological function of the vanadium.

The first phase of these studies culminated in the work of Webb (1939) who was able to correlate the presence of vanadium in ascidians with certain evolutionary traits of the class. Webb concluded that vanadium is concentrated by the primitive species and that this enrichment ability has been lost in the later, more specialized, branches of the class.

Webb also systematically studied the physiological role of vanadium. He demonstrated that the green chromogen containing the vanadium in some species is located in a singular type of blood cell which he called the "vanadocyte." Later, however, in histological investigations on certain Japanese ascidians, Ohuye (1936) described what may be the same type of green cells in these animals which, according to the analyses of Kobayashi (1948), contained no vanadium. Webb found no physiological function for the vanadium, although previously it had been assumed to be associated with a blood respiratory pigment. He also postulated that the vanadium was incorporated in a rectilinear chain of pyrrole rings rather than in a protein or porphyrin complex.

The reported distribution of the vanadium within ascidians is somewhat contradictory. Webb suggested that all of the vanadium is located in the blood, whereas the experiments of Kobayashi and of Bertrand localized the majority of the vanadium in other parts of the animal. In *Ascidia mentula* Bertrand found ⅔ of the vanadium in the tunic, yet Kobayashi was able to establish only traces of vanadium existing in the outer covering of Japanese ascidians.

The dynamics of the concentration of vanadium from sea water by these marine animals has received scant attention. Bertrand describes the situation as a complete mystery and states that the vanadium could come neither from sea water, which is very poor in vanadium, nor from vanadium-impoverished marine muds in the vicinity of the ascidians. He invites research on plankton as a potential source of vanadium. Rankama and Sahama (1950) suggest, however, that the vanadium is collected by ascidians directly from sea water.

Thus, from a biogeochemical standpoint it seemed desirable to investigate the more intimate details of the concentration of vanadium by marine organisms. The

[1] Contributions of the Scripps Institution of Oceanography, New Series No. 528.

[2] A comprehensive survey of the biogeochemistry of vanadium has been prepared by D. Bertrand (1950).

present investigation was undertaken in order to attempt to follow the transfer of vanadium from the sea into the various parts of the body of ascidians. A knowledge of the mode of uptake and its histological distribution might shed light upon a possible role played by the vanadium.

To lay the groundwork for these investigations, chemical analyses of dissected portions of some local ascidians were made. This work was intended to establish more definitely the sites of vanadium concentration. A limited number of analyses were made on the total vanadium content of some local species for inclusion in Webb's phylogenetic table.

Cyclotron-produced radioactive vanadium, free from any inert vanadium carrier, was utilized to follow the assimilation of the element. Radioautographs were made to confirm the results of the chemical analyses and also to point out any smaller loci of vanadium concentration not detected by the less sensitive chemical method.

The authors wish to express their appreciation for the aid given by Dr. Denis L. Fox, Professor of Biochemistry at the Scripps Institution of Oceanography, for his help in the completion of this problem.

Chemical Analytical Techniques

Specimens of *Ciona intestinalis* were collected from floats in Mission Bay, San Diego, California. The *Ascidia ceratodes* specimens were obtained from dredgings in Tamales Bay, Marin County, north of San Francisco, California.

Table I

Vanadium content of Ciona intestinalis

Specimen A			Specimen B		
Sample	Dry Wt.	Vanadium	Sample	Dry Wt.	Vanadium
	grams	γ		*grams*	γ
Tunic	0.1629	0.0	Tunic	0.3317	0.0
Mantle	—	—	Mantle	0.0709	0.0
Ovary	0.0330	2.5	Ovary	0.0241	0.0
Heart	0.0084	0.0	Heart	0.0113	0.0
Incurrent Syphon	—	—	Incurrent Syphon	0.0368	4.0
Excurrent Syphon	—	—	Excurrent Syphon	0.0228	0.0
Esophagus	—	—	Esophagus	0.0014	0.0
Branchial Basket	—	—	Branchial Basket	0.0241	0.0
Gut			Gut		
Anterior Half	0.0517	19.7	Anterior Half	0.1116	21.9
Posterior Half	0.1375	8.9	Posterior Half	0.1252	37.8
Stomach	0.0543	18.8	Stomach	0.0586	9.2
Rest of Specimen and Rinse	0.2299	27.0	Rest of Specimen and Rinse	—	—
Rinse		—	Rinse	0.2955	19.6
Total	0.6777	76.9	Total	1.1140	92.5
Date Ciona Obtained	24 July 1950		Date Ciona Obtained	20 April 1950	
Wet Weight	19.0 grams		Wet Weight	19.0 grams	
Length	8.0 cm		Length	6.5 cm	

TABLE II

Total vanadium contents of some ascidians

Ascidian	Parts Vanadium Per Million
Styella montereyensis	0
Styella bonharti	0
Euherdmania claviformis	475
Ciona intestinalis	100

The specimens were cleaned of adhering dirt and sessile organisms by the light application of a tooth brush. After dissection the individual samples were placed in weighed 50 ml. Pyrex centrifuge tubes. The tube and contents were dried overnight at 110° C., cooled in a desiccator, and weighed to determine the "dry weight" of the sample.

The organic matter was removed by a wet digestion as follows: to the sample-containing tube, 3.0 ml. of a solution of one part concentrated sulfuric acid to three parts of 60 per cent perchloric acid were added. The tube was heated gently with a micro-burner until the sample was nearly completely digested. Stronger heat was then applied to facilitate the complete removal of the perchloric acid. The sulfuric acid residue was diluted to 40 ml. with distilled water, and any remaining solids (usually diatom frustules) were removed by centrifugation.

The vanadium in the clear solution was determined by a spectrophotometric analysis of the vanadium 8-hydroxyquinolate in iso-amyl alcohol according to the method of Bach and Trelles (1941). The samples were read at 480 mμ in 50 mm. cells on a Beckman Model DU Spectrophotometer.

The results of two typical analyses of *Ciona intestinalis* are summarized in Table I. The vanadium concentrations for other ascidians collected during the course of these investigations are listed in Table II.

Relatively high concentrations of vanadium occur in the gut. The gut tube was arbitrarily dissected into four serial lengths. It was found that the highest vanadium concentrations per unit weight were associated with that part of the gut nearest the stomach, and with the segment adjoining the anus (Table III). Vanadium was normally detected in the stomach by these chemical analyses.

Vanadium was also found in fecal pellets in concentrations ranging from 0 to 800 parts per million by weight. Fecal pellets discharged by the animal during its relaxation before dissection, or dislodged during the dissection, may account for the high vanadium content of the rinse water as noted in Table I.

TABLE III

Concentration of vanadium in gut sections of Specimen B

Section	Length	Dry Weight	Vanadium
1 Next to Stomach	10 mm.	0.0601 gr.	19.3γ
2 Next segment	10 mm.	0.0515 gr.	2.6γ
3 Next segment	10 mm.	0.0601 gr.	11.8γ
4 Next to Anus	10 mm.	0.0651 gr.	26.0γ

Feeding Experiments Utilizing Radioactive Vanadium

V^{48}, a positron and gamma ray-emitting nuclide with a half-life of sixteen days, was prepared by the deuteron bombardment of a titanium target according to the reactions $Ti^{47}(d,n)V^{48}$ and $Ti^{48}(d,2n)V^{48}$. The bombardments were made at the 60-inch Crocker Cyclotron in Berkeley, California, with 20 Mev deuterons for one hour with a total irradiation of 7 micro-ampere hours. The titanium target was composed of one gram of the C.P. metal powder supported on a copper plate covered with a 0.25 mil tantalum foil.

The sub-micro amounts of V^{48} were extracted from the titanium powder to give a carrier-free saline solution of radioactive vanadate, following the method of Haymond, Maxwell, Garrison and Hamilton (1950). The nuclide V^{48} was identified by its half life and by aluminum absorption curves to establish the 0.7 Mev positron reported by Peacock and Deutsch (1946).

Individual ascidians were then placed in aerated Pyrex containers which held two liters of sea water. These aquaria were kept in a cold room maintained at a temperature of $12° \pm 2°$ C.

The sea water was normally filtered before use. In preliminary experiments it was found that the radioactive vanadium was adsorbed on particulate matter in non-filtered sea water and was thus removed from solution. The water was filtered through either Whatman No. 42 paper or a diatomaceous earth column. The radioactive vanadium was added after filtration.

The activity of the solution was assayed daily by evaporating under an infrared lamp a one-ml. aliquot of the sea water in a steel cup. The activity of the sample was counted under an end-window tube with a window thickness of 1.4 mg./cm^2.

A control aquarium was set up without an ascidian to determine whether there was adsorption of the radioactive vanadium on the walls of the container. No measureable adsorption was found to take place.

The initial counting rate of the radioactive solution was about 250 counts per minute per ml. of solution. The counter geometry was estimated at 5 per cent for 0.7 Mev positrons from measurements with a calibrated U_3O_8 source and aluminum absorption experiments on the active vanadium. Utilizing the radioactive decay equation,

$$dN/dt = -\lambda N \qquad (1)$$

where N is the number of atoms of the radionuclide present, λ is the decay constant equal to 0.693 divided by the half life, and dN/dt is the instantaneous disintegration rate, 10^{11} atoms of active vanadium are found to exist in one liter of solution initially. This may be compared with the upper limit of vanadium in sea water of 10^{16} atoms per liter as determined spectroscopically by D. Bertrand (1942). It should be noted, however, that the experimentally determined impurity of 0.01 per cent of vanadium in the one gram of titanium metal target introduced about 10^{16} atoms of vanadium when it was diluted to 100 liters in the experiments that were undertaken.

Three possibilities existed for the mode of removal of vanadium from sea water by the ascidians. The first type would be an erratic assimilation in which no consistent variation of the remaining vanadium in the solution with time would be found. In the second case, the ascidians might concentrate constant amounts of

vanadium per unit time, whereby a plot of the vanadium remaining in solution against time would give a linear relationship. Another possible mechanism would involve the ascidians concentrating the vanadium in direct proportion to the amount of vanadium present in the sea water environment. In such a case, a graph of

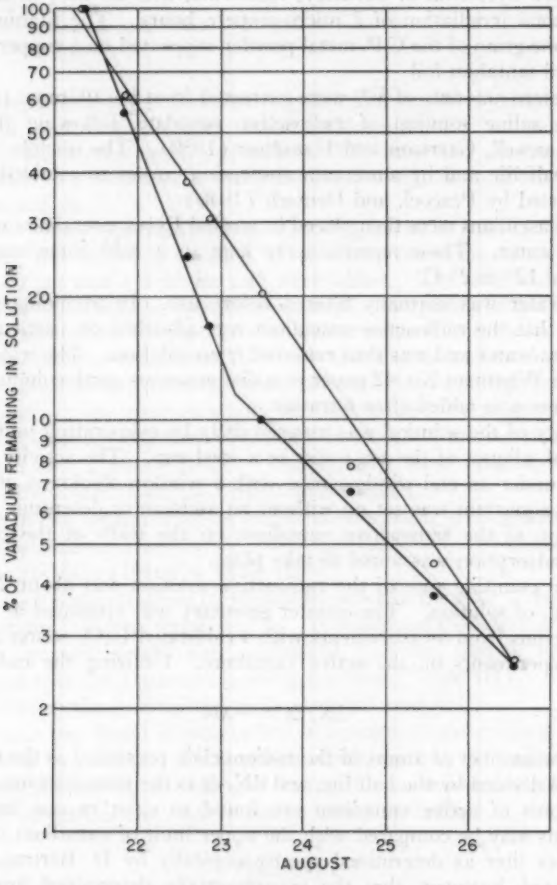

FIGURE 1. Two uptake curves of vanadium by *Ascidia ceratodes*. ○ Experiment 101. ● Experiment 2.

the logarithm of the remaining amount of vanadium in the solution against time should result in a linear relationship. This last mechanism was found to be followed in experiments with *Ascidia ceratodes*, as is illustrated in Figures 1 and 2 where the logarithm of the vanadium remaining in solution (corrected for radioactive decay) is plotted against the time of withdrawal of the aliquot of sea water. In

studies on *Ciona intestinalis* all of the curves obtained could be divided into two straight lines wherein a high initial uptake was followed by an abrupt change to a lower feeding rate. This is illustrated in Figure 2. In 10 experiments on *Ascidia ceratodes,* only two sharp changes were found in each case.

The instantaneous uptake of vanadium by these ascidians may be designated by

$$dN/dt = - VR \qquad (2)$$

where V is the concentration of vanadium at time t in the sea water, N is the total amount of vanadium in the volume A of the sea water, and R is the effective clearance rate of the vanadium from the solution in units of volume per unit time,

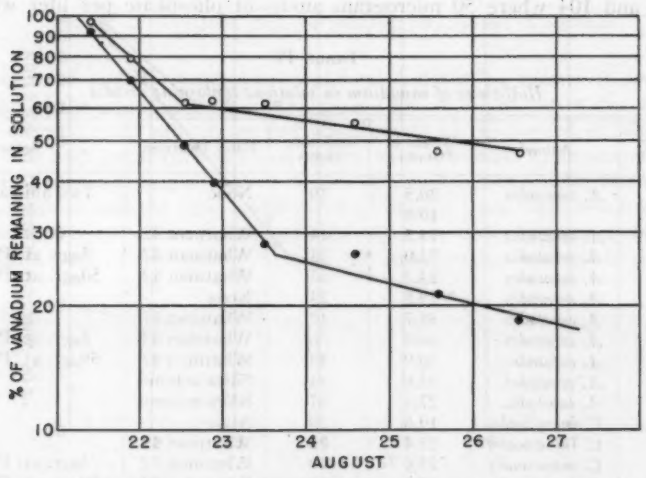

FIGURE 2. Two uptake curves of vanadium by *Ciona intestinalis.* ○ Experiment 7. ● Experiment 5.

assuming a 100 per cent uptake of vanadium. It follows that

$$V = N/A. \qquad (3)$$

Utilizing Equation (3) and integrating Equation (2) from the beginning of the experiment we arrive at

$$V/V_0 = e^{-(R/A)t} \qquad (4)$$

where V_0 represents the initial concentration of vanadium. For the purpose of comparative study we define the "half-time of vanadium in solution" as the time required for a given animal to reduce the vanadium concentration to half of its initial value. The values for the half-time for the 14 experiments conducted are listed in Table IV with the details of each run. Where changes occurred, the initial half-time is given.

The half-time will be a function of the effective adsorbing surface (*i.e.,* the geometry) and the rate at which the water is circulated through the animal. Where

there is a change in the half-time during the course of an experiment, either or both of these parameters may change.

These experiments indicate that the initial transfer of vanadium from the hydrosphere to ascidians occurs by an adsorption process where the adsorbing surface is in an unsaturated state with respect to the adsorption of vanadium at all times, or is maintained in such a state by a constant regeneration of the surface. In either case the number of vanadium ions (or colloidal particles containing vanadium) would determine the number adsorbed at any given time.

To study more closely the dynamics of the vanadium transfer, the sea water in six experiments was enriched in phosphate ion. In the case of *Ascidia ceratodes* a high phosphate level inhibited the uptake of vanadium. This is seen in Experiments 4 and 104 where 50 microgram atoms of phosphate per liter were added

TABLE IV

Half-times of vanadium in solutions containing ascidia

Experiment	Animal	Weight in Grams	Half-time Hours	Filter Treatment	Remarks
1	*A. ceratodes*	20.5 10.0	10	None	Two animals
2	*A. ceratodes*	13.5	14	Whatman 42	
3	*A. ceratodes*	23.0	24	Whatman 42	5μgr. at. $PO_4^{\equiv}$/liter
4	*A. ceratodes*	23.2	65	Whatman 42	50μgr. at. $PO_4^{\equiv}$/liter
101	*A. ceratodes*	29.8	24	None	
102	*A. ceratodes*	38.7	12	Whatman 42	
103	*A. ceratodes*	36.8	6	Whatman 42	5μgr. at. $PO_4^{\equiv}$/liter
104	*A. ceratodes*	30.9	48	Whatman 42	50μgr. at. $PO_4^{\equiv}$/liter
9	*A. ceratodes*	35.0	14	Silica column	
10	*A. ceratodes*	27.1	37	Silica column	
5	*C. intestinalis*	19.6	29	None	
6	*C. intestinalis*	25.4	22	Whatman 42	
7	*C. intestinalis*	25.6	40	Whatman 42	5μgr. at. $PO_4^{\equiv}$/liter
8	*C. intestinalis*	47.6	18	Whatman 42	50μgr. at. $PO_4^{\equiv}$/liter

initially to the sea water. In these two experiments the greatest half-times were observed. Five microgram atoms of phosphate per liter had little effect upon the uptake as is noted in Experiments 3 and 103. Since pentavalent vanadium ion can resemble phosphate ion chemically in solution, these experiments indicate that the phosphate ion competed successfully with the vanadium in the uptake process, and that vanadium in an ionic form rather than a colloidal vanadium is adsorbed. However, the solution chemistry of elements in concentrations in the level of vanadium in the sea and in our experiments has not been developed to the extent to give an unqualified answer.

PUMPING RATE STUDIES ON CIONA INTESTINALIS

To determine the efficiency of transfer of the vanadium from the sea water to the animals, investigations concerning the effective pumping rates of *Ciona intestinalis* were conducted. In each experiment a single animal was placed in a small plastic aquarium two inches deep (Fig. 3). A continuous flow of sea water

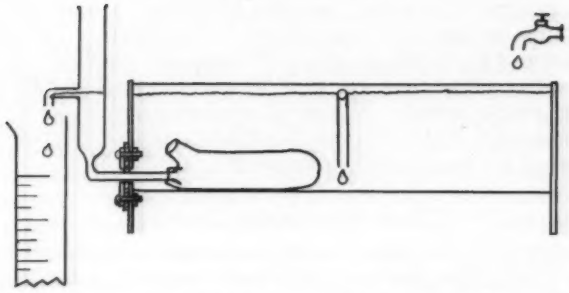

FIGURE 3. Aquarium used for pumping rate studies of ascidians.

was maintained and the water level was kept constant by means of drains placed at the top of the aquarium. A Pyrex glass tube of 4 mm. outer diameter with a 6 mm. outer diameter bulb at one end was inserted in the opened exhale syphon of the ascidian with great care to avoid injury to the animal. The animals soon relaxed with such an arrangement. The ascidian, with inserted tube, was then placed in the aquarium and the tube connected to a second glass tube which entered the aquarium through a rubber gasket and discharged the water outside of the aquarium at the same level as the drains. There was therefore no hydrostatic head to aid

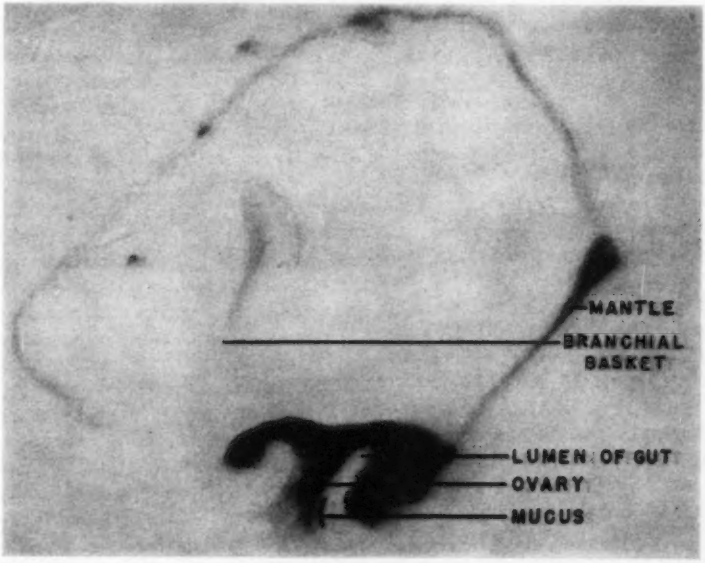

FIGURE 4. Radioautograph of *Ascidia ceratodes* used in Experiment 9. Concentration of vanadium is seen in ovary, gut wall, and in the mucus in the gut. Fifteen day exposure.

or hinder the ascidian in its normal pumping functions. The animals pumped at a constant rate after the tube had been inserted for two hours. The animals seemed to seal their junction to the tube by means of mucus and muscular tonus.

For an ascidian with a wet weight of 25 grams, pumping rates of about two liters per hour were observed. This result compares favorably with the pumping studies of Jørgensen (1949) on *Ciona intestinalis*; using a different technique, he obtained values of the same order of magnitude.

Radioautographic Studies

At the completion of the uptake studies with radio-vanadium the animals were sacrificed for radioautographic analysis. Both *Ascidia ceratodes* and *Ciona intestinalis* were fixed in Bouin's solution, imbedded in paraffin, and sectioned at about 100 micra. Survey autographs were obtained with Eastman No-Screen X-Ray film with exposure times up to 30 days. Figures 4 and 5 show two typical transverse sections.

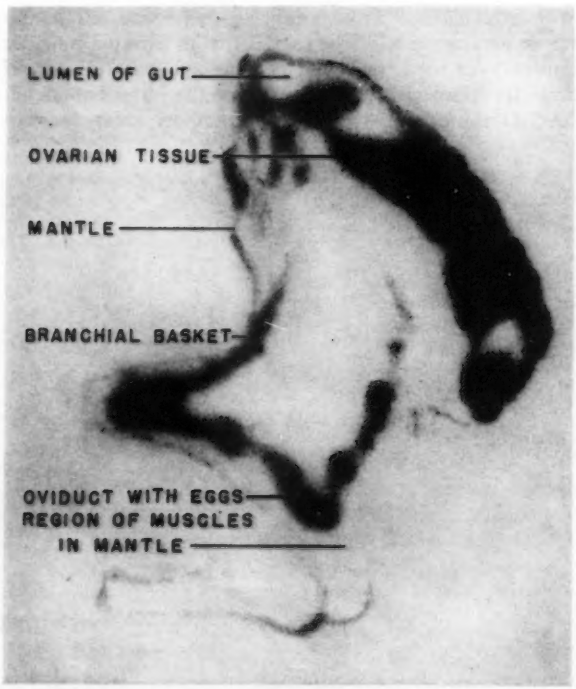

Figure 5. Radioautograph of *Ascidia ceratodes* used in Experiment 102. Concentration of vanadium is seen primarily in ovary, gut wall, oviduct (eggs), and branchial basket, with very small amounts in the mantle. No vanadium concentration was found in the muscles of the mantle. Exposure time of 15 days.

The autographs confirmed the chemical analyses on *Ciona intestinalis*, showing high concentrations in the gut wall and in the ovary, and showed similar local concentrations in *Ascidia ceratodes*. The vanadium concentration in the ovaries of both species is primarily in the follicles. The vanadium was still in the mature eggs when they were extruded from the ovary into the peribranchial cavity.

There is some concentration of the vanadium in the branchial basket of both species, though sometimes not in very great amounts. This enrichment may be due to the blood vessels in this structure, as high concentrations of vanadium in blood vessels were observed. The mantle and test showed very little vanadium and the muscles of the mantle displayed no evidence of this element. Vanadium appeared in the mucus of the branchial basket and in an isolated bit of mucus in the gut as is shown in Figure 4. The absence of high amounts of vanadium in the branchial region may have been due to washing away of the mucus sheet during the fixing procedure or to the constant renewal of fresh mucus by the living animal.

Discussion

The uptake experiments establish that vanadium can be concentrated directly from solution by the two species of ascidians used in this work. To obtain an idea of the efficiency of this adsorption process, we may compare the effective clearance rate of vanadium with the pumping rates for *Ciona intestinalis*. By substitution in Equation (4) of A = 2 liters/hour, $V/V_0 = \frac{1}{2}$, and the half time = 30 hours (the average from Experiments 5, 6, and 7), we arrive at an effective clearance rate of vanadium of 0.05 liters per hour. Compared with the observed pumping rate of 2 liters an hour, the efficiency of uptake of vanadium is of the order of 2½ per cent. This is the value for animals which were unfed, since food in the form of particulate matter could not be introduced without adsorbing vanadium from solution and invalidating the results.

The extraordinary biological specificity of the adsorbing surface of the ascidians for the vanadium in aqueous solution remains an unsolved problem of high biochemical interest. This selectivity is undoubtedly related to the unique ionic structure and properties of vanadium. The mucus sheet fulfills the requirement of a constantly renewed surface. The high vanadium content of the mucus, as shown by the autographs, in the branchial basket and in the gut, points to this sheet as the adsorbing surface. The sharp discontinuities in the uptake curves may be explained by chemical or area changes in the adsorbing surface.

The high concentrations of vanadium in the ovarian follicles, as shown in the autographs, suggest that this element may become part of a metabolic system that is present from the beginning in the developing embryo. Further work utilizing more concentrated radioactive vanadium solutions and thinner autograph specimens should reveal more on the intimate histology of vanadium in these animals.

We may now attempt to account for the high amounts of vanadium found in ascidians. In the case of *Ciona intestinalis* which contains about 100 micrograms of vanadium in a mature 20 gram animal, a minimum of 450 liters of sea water may be effectively cleared of vanadium in a year. This is equivalent to about 45 micrograms of vanadium. Further, we must consider that vanadium and many other elements are ionically or colloidally adsorbed on the particulate matter of the oceans. This phenomenon presented itself in the preliminary feeding experiments.

Thus, a more concentrated source of vanadium is available to these filter feeders. A final potential store of vanadium lies in the plankton of the oceans, a source of ultimate food for these filter feeding animals.

Summary

1. Chemical analyses made on *Ciona intestinalis* indicated the vanadium to be localized in the gut region and the ovaries. These sites of assimilation were confirmed by radioautographs utilizing cyclotron-produced radioactive vanadium.

2. The total vanadium content of four local species of ascidians was determined. The uptake of radioactive vanadium from sea water indicated that *Ciona intestinalis* and *Ascidia ceratodes* are able, by means of an adsorption mechanism, to concentrate vanadium directly from sea water. The efficiency of assimilation was of the order of 2½ per cent for unfed animals.

3. Pumping rate of water by *Ciona intestinalis* was determined by a direct method. It was concluded that sea water and its particulate constituents could furnish the necessary vanadium demanded by *Ciona intestinalis*.

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RELATIONSHIP BETWEEN NUCLEAR VOLUMES, AMOUNT OF INTRANUCLEAR PROTEINS AND DESOXYRIBOSENUCLEIC ACID (DNA) IN VARIOUS RAT CELLS

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The concept that increase in size of nuclei and cells within one tissue is directly correlated with an increase in chromosome number or chromosomal mass has become more or less accepted in cytology (Hertwig, 1934, 1939).

But Montgomery (1910), Bowen (1922) and Schrader (1947) have shown that in different sizes of spermatocytes in pentatomid testes, the chromosome number and chromosome size are identical in the different sized cells. Recently, Schrader and Leuchtenberger (1950), using quantitative cytochemical methods, presented evidence that in the pentatomid Arvelius there exists a direct correlation between three sizes of nuclear volumes of spermatocytes (200, 400, 1600 cubic microns), and the amount of contained proteins, while the amount of DNA was the same in these nuclei. On the basis of these results, the assumption was made that changes in nuclear sizes in a tissue are due mainly to alterations in protein and water content, and not due to different amounts of DNA.

The present communication presents further evidence on a parallelism between size of nuclei and amount of intranuclear protein within one tissue, but points out that such a correlation does not apply to a tissue containing cells with different synthetic activities, such as the parietal and chief cells of the gastric mucosa.

Material and Methods

Rat tissue was fixed immediately after death in 50 per cent formalin and Carnoy and cut at different thicknesses, according to the requirements of the investigation. The estimation of DNA and protein was done by the cytochemical and photometric microscopic methods previously described (Schrader and Leuchtenberger, 1950; Pollister, 1950).

Results

As can be seen from the data in Table I, there occur three distinct classes of DNA, namely 3.3, 6.6 and 12.8, in liver nuclei of the rat, an observation which is in agreement with the findings of Ris and Mirsky (1949) and Leuchtenberger, Vendrely and Vendrely (1951). But it is also evident that in contrast to the nearly exact multiples of DNA in the nuclei (1, 2, 4), the nuclear volumes do not show such a multiple relationship, but vary over a large range with intermediate values. It can be furthermore seen that there is no correlation between nuclear size and amount of DNA per nucleus; within each DNA class the size of nuclei varies considerably. For instance, among 36 nuclei with a DNA content of 6.6 per nucleus,

TABLE I

Relationship between nuclear volumes, amounts of intranuclear proteins and desoxyribosenucleic acid of rat liver cells*

	Mean Amount of DNA per Nucleus = 3.3 ± 0.08			Mean Amount of DNA per Nucleus = 6.6 ± 0.24			Mean Amount of DNA per Nucleus = 12.8 ± 0.50		
Mean nuclear volumes in cubic microns	62.0	153±17	289±27	285±20	455±30	732±78	376	637	1138
Number of nuclei measured	3	20	6	18	13	5	4	4	6
Mean amount of protein per nucleus	1.9	4.6±0.23	8.7±0.3	8.6±0.1	13.7±0.5	22.0±1.2	11.3	19.0	34±1.4

* Amounts expressed in arbitrary units.

18 showed a nuclear volume half or less than that of the remaining nuclei. The independence of nuclear size and of DNA content is also obvious if one compares the nuclear volumes of one DNA class with those of another DNA class; *i.e.*, 6.6 DNA per nucleus occurs in cells with nuclear volumes which are as small as those of the 3.3 and as large as those of the 12.8 DNA class. The occurrence of nuclear volumes in nonmultiples is in agreement with the data of Biesele (1944) on volumes of rat liver nuclei.

The picture is considerably different if one considers the amount of protein per nucleus in relation to nuclear size. It is evident that there exists a direct correlation between both; any increase in nuclear volume is accompanied by a corresponding increase in amount of intranuclear protein. In order to see whether such a close relationship between size of nuclei and amounts of contained proteins would hold in tissues in which cells with different functions occur, we examined the gastric mucosa of the rat. It is known that there the chief cells produce a protein, namely a precursor of pepsin, while the parietal cells produce HCl. In the following Table II, the results are presented.

On the basis of the data in Table II it is evident that the amount of DNA per nucleus is the same in chief cells and parietal cells. It can be further seen that

TABLE II

Relationship between nuclear volumes, amounts of intranuclear proteins and desoxyribosenucleic acid of rat gastric mucosa cells*

Type of Cell	No. of Nuclei Measured	Nuclear Vol. in Cubic Microns	DNA per Nucleus	Mean amount of Protein per Nucleus
Parietal Cell	19	57±4	2.9±0.1	1.0±0.1
Chief Cell	20	44±2	3.1±0.1	1.9±0.09

* Amounts expressed in arbitrary units.

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though the nuclear volumes of the parietal cells are larger than those of the chief cells, the latter contain a larger amount of protein per nucleus than the parietal cells. Therefore, the strict parallelism between amounts of proteins and nuclear size, as found in liver cells or spermatocytes, does not necessarily hold for cells which differ in the nature of their synthesizing activities, such as the chief and parietal cells of the gastric mucosa.

Discussion

The close correlation between amount of intranuclear proteins and nuclear volumes, and the independence of the amount of DNA from the latter in rat liver nuclei is in good agreement with the previous work of Schrader and Leuchtenberger (1950) concerned with proteins and DNA in spermatocytes.

A similar correlation between amount of proteins and nuclear sizes in oocytes of mice was recently reported by Alfert (1950). The striking parallelism between amounts of proteins and size of nuclei in all these tissues is based on the fact that the concentration of proteins stays the same in all nuclei, whether the cell decreases or increases in size.

But these observations do not apply to all the tissues of the same animal, as can readily be seen from the results in cells of the gastric mucosa. It seems that the correlation exists only if cells actually have the same metabolic function as, for instance, the cells of the parenchyma of liver or the spermatocytes in the testes. However in a tissue in which cells occur having different synthetic activities, the concentration of the proteins may vary. In the gastric mucosa, the protein-producing chief cells show nearly twice the amount of protein per nucleus, though their nuclear volume is smaller than that of the HCl-producing parietal cells. These results are in good agreement with Caspersson's work (1950), who, on the basis of ultraviolet studies, reported considerably larger amounts of proteins and ribosenucleic acid in chief cells than in the parietal cells.

Whether it can be assumed generally that variable concentrations of proteins in different cells of the same tissue indicate differences in protein synthesis cannot be decided at this time. But the possibility is now opened that the cytochemical photometric determination of proteins in individual cells may allow such a differentiation.

A comparative study of the protein content of nuclei of normal and abnormal cells is now in progress.

Summary

Cytochemical photometric microscopic determinations of proteins and desoxyribosenucleic acid in cells of rat tissues gave the following results:

1. A direct correlation exists between different sizes of nuclei and amounts of intranuclear proteins in rat liver cells, while the amount of DNA was found to be independent of nuclear size.

2. In the gastric mucosa, where cells with different synthetic activities (parietal and chief cells) occur, there is no parallelism between nuclear size and proteins. The chief cells have a larger amount of protein than parietal cells, while the amount of DNA is the same in both.

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ACTION OF VARIOUS ENZYMES ON THE MATING TYPE SUBSTANCES OF PARAMECIUM CALKINSI[1]

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When paramecia of complementary mating type are mixed under appropriate conditions, these animals adhere to one another and shortly form large clumps or agglutinates. All available evidence (Kimball, 1943; Metz, 1948) indicates that this initial agglutinative mating reaction results from interaction of complementary substances or at least complementary configurations on the surfaces of the cilia. For convenience these substances or configurations are called the mating type substances.

Recent studies have shown that interaction of mating type substances may perform a far more significant function in fertilization than the obvious one of effecting the initial union of potential conjugants. Thus, Metz and Foley (1949) using a mutant stock of *Paramecium aurelia* found that only the initial mating reaction union is essential for induction of meiosis, macronuclear breakdown, loss of mating reactivity and pseudo selfing pair formation in this species. Since the mating reaction alone was essential for induction of these activation phenomena, it seemed reasonable to conclude that interaction of mating type substances initiates the activation. In view of this, it is of interest to obtain more specific information regarding the physical basis for the mating reaction, in other words to define the mating type substances in more concrete physico-chemical terms. In an attempt to achieve this goal an investigation of the chemical nature of the mating type substances has been undertaken. As one phase of this study several enzymes were tested for action on the mating activity of paramecium. *Paramecium calkinsi* was selected for this study because of the ease with which cultures of this species may be obtained in mating condition. Of the several enzymes tested, only the proteolytic enzymes, trypsin and chymotrypsin, affected the mating reactivity of the paramecia.

Material and Methods

Enzyme preparations. The following enzyme preparations were tested for their effects on the mating reactivity of *P. calkinsi*: crystalline preparations (Armour) of trypsin, chymotrypsin, lysozyme and ribonuclease; purified hyaluronidase and crude preparations of ptyalin and lecithinase (bee venom, prepared by the method of Flury, 1920).

Preparation of the paramecia for testing. As pointed out elsewhere (Metz and Butterfield, 1950), paramecia in mating condition quickly lose their mating reactivity when fed bacteria. Furthermore, paramecia appear to feed upon concentrated protein solutions (enzymes, rabbit sera) since they enlarge, become filled with food vacuoles and sometimes divide in such solutions. To avoid the possibility of such

[1] Aided by a grant from the National Institutes of Health, U. S. Public Health Service.

indirect loss of mating activity from feeding, the paramecia were killed with formalin or picric acid before treatment with enzyme. Paramecia killed by appropriate treatment with these agents give strong, specific mating reactions. Indeed, such dead animals can activate living animals of opposite type (Metz, 1948). The reader is referred to the article of Metz and Butterfield (1950) for the methods of culturing and killing the *P. calkinsi*.

Method of treatment with enzyme. To test for action on the mating substance, both the enzyme preparation and the formalin- or picric acid-killed reactive animals were taken up in an appropriate buffer with pH adjusted near the optimum for enzyme action. Equal volumes of enzyme solution and paramecium suspension were then mixed in depression slides or precipitin tubes. After an appropriate incubation period (1–6 hrs., 20°–30° C.) the treated animals were washed free of enzyme solution with ⅔ sea water and tested for mating reactivity with living reactive type I and II animals. To control for reactivity of the dead animals and to check for inactivation of the animals by the buffer, equivalent samples of the same dead paramecia were treated with buffer but without enzyme solution. When appropriate known enzyme substrates were available, these were employed as controls for enzyme activity. To accomplish this, samples of the enzyme solution and a solution of appropriate known substrate in the same buffer were mixed and incubated in parallel with the treated paramecia. In a few cases the experiment was set up in such a manner that the enzyme digested an amount of known substrate equal to or greater than the mass of the enzyme treated paramecia. The mass of the paramecia was determined roughly by counting the animals present and multiplying by 0.05, the weight (in micrograms) of one *Paramecium calkinsi*. This figure was obtained from the number (7.5×10^5) of paramecia in a 37.3 mg. sample of washed, lyophylized animals. It agrees reasonably well with the figures of Boell and Woodruff (1941).

As a final control, the supernatant buffer from a sample of the dead paramecia usually was tested for inhibition of enzyme action. Conceivably substances leaching from the dead animals could inhibit the enzyme in the test sample. Traces of the formalin or picric acid used to kill the paramecia would fall into this category. In all cases where this control was employed, the enzyme was active in the animal supernatants.

Results

Non-proteolytic enzymes. Crystalline (Armour) preparations of lysozyme and ribonuclease, purified (1300 turbidity reducing units/mg.) hyaluronidase and crude preparations of ptyalin and lecithinase (bee venom) all had no detectable effect upon the mating reactivity of dead *P. calkinsi*. The conditions under which each of these enzymes was tested are given in Table I.

Proteolytic enzymes. In marked contrast to the preparations considered above, the proteolytic enzymes trypsin and chymotrypsin destroyed the mating reactivity of formalin- or picric acid-killed *Paramecium calkinsi* of both mating types. Five-tenths per cent crystalline (Armour) trypsin and chymotrypsin solutions were made up in borate buffer, pH 8. Equal volumes of these enzyme solutions and suspensions of dead paramecia in the same buffer were mixed and allowed to stand. At intervals samples of dead animals were removed from the enzyme solution, washed in ⅔ sea water and tested for mating reactivity with living type I and II test

animals. A portion of each sample was also stained with iodine solution and examined under the oil immersion objective for the condition of the cilia. Dead animals from the same original sample but treated with buffer only (no enzyme) served as the standard of comparison both for mating reactivity and condition of the cilia. The results of a typical experiment with trypsin and chymotrypsin are given in Table II. It is evident from the data of Table II that the trypsin and chymotrypsin preparations destroy the mating reactivity of both type I and II animals.

The striking difference in time for inactivation of formalin-killed as opposed to picric acid-killed animals is not readily explained. Possibly this difference in in-

TABLE I

Conditions under which non-proteolytic enzymes were tested for action on formalin- and picric acid-killed Paramecium calkinsi

Enzyme	Animals Treated*	Buffer	pH	Final Enzyme Concentration	Temperature	Digestion Time	Inhibition of Enzyme by Supernate	Mg. Animals Treated with Enzyme	Mg. Known Substrate Digested
Bee venom	F I	2.5 sea water	6.9	0.63%	28° C.	1.5 hrs.	No inhibition	0.045	Hemolyzed 2% RBC suspension
Hyaluronidase[2]	F I	2.5 sea water	6.9	0.63%	28° C.	2 hrs.		0.018	
Lysozyme	F I	0.0025 N NaCl	6.6	0.5%	Room temp.	2.7 hrs.	No inhibition	>1.0	Lysis of turbid *S. lutea* suspension
	P I	NaCl	6.6	0.5%	Room temp.	2.6 hrs.		>1.0	
	P II	NaCl	6.6	0.5%	25° C.	2.0 hrs.		>1.0	
Ptyalin	F I, P II	Phosphate	6.6	1/2 sat. sol.	37° C.	2.0 hrs.	No inhibition	0.4	0.6 mg. soluble starch
Ribonuclease[3]	F I	Borate	8.0	0.075%	30° C.	6 hrs.	No inhibition	0.1 mg. paramecium nitrogen	0.7 mg. Na nucleate nitrogen >5 mg. Na nucleate
	P I, P II	Borate	8.0	0.05%	Room temp.	6 hrs.			

* F I indicates formalin-killed type I animals; P I, picric acid-killed type I animals; and P II, picric acid-killed type II paramecia.

[2] The authors are indebted to Dr. J. Seifter, Wyeth Institute of Applied Biochemistry, for this preparation of hyaluronidase.

[3] The ribonuclease preparation was found to hydrolyze casein. This proteolytic action was removed by the method of McDonald (1948).

activation time simply reflects a difference in the number of reactive groups exposed on the surfaces of the paramecia. This view is supported by the fact that formalin-killed animals usually give stronger mating reactions than picric acid-killed animals. Possibly traces of formalin leaching from the dead animals inhibit the enzymes to a greater degree than corresponding traces of picric acid. Finally, differences in the extent of alteration ("denaturation") of paramecium proteins by formalin, as opposed to picric acid, may account for these results. In this connection it will be recalled that denatured proteins are known to be more susceptible to the action of proteolytic enzymes than the native material (Haurowitz, 1950).

As might be expected, the enzyme preparations not only destroy the mating reactivity of the dead animals but in time they digest the cilia and eventually reduce

the formalin- or picric acid-killed animals to fragile ghosts. However, the enzyme-treated dead animals can lose their ability to give the mating reaction before the cilia show any appreciable action of the enzyme. Cilia of control formalin- or picric acid-killed paramecia are usually straight and extend in a radial direction from the body of the animal. After treatment with trypsin or chymotrypsin, however, the cilia (iodine stained) gradually lose sharpness of outline, become curly and finally disappear. The picric acid-killed animals of Table II still possessed cilia after a 17 minute treatment with trypsin. However, the cilia of these animals could be distinguished from those of the controls. The former were classified as "fair to good" (picric acid-killed type I animals at 17 minutes) or "poor to fair" (picric acid-killed type II animals) when compared for sharpness of outline and straightness with the controls. Likewise, picric acid-killed, chymotrypsin-treated animals retained their cilia for some time following loss of mating activity. Even at 70 minutes these type I animals (Table II) possessed fair to good cilia. The cilia of some animals were indistinguishable from those of controls. At 165 minutes,

TABLE II

Mating reactivity of trypsin- and chymotrypsin-treated dead Paramecium calkinsi

Enzyme	Digestion Time	Enzyme Treated			Buffer Treated		
		Formalin-killed I	Picric Acid-killed		Formalin-killed I	Picric Acid-killed	
			I	II		I	II
Trypsin	17 min.	++++	—	—	++++	++-+++	+++
	62 min.	++	—	—	++++	++	++
	137 min.	++	—	—	++++	++	++-+++
Chymo-trypsin	25 min.	+++	—	+	++++	++	+++
	70 min.	++	—	—	++++	++	+++
	165 min.	++	—	—	++++	++	++

however, these animals were devoid of cilia. The picric acid-killed type II animals had "poor to fair" cilia after 60 minutes treatment with chymotrypsin and no cilia after 165 minutes treatment.

From these and other observations on trypsin and chymotrypsin treated animals, it is evident that loss of mating activity does not necessarily involve any serious visible change in the structure of the cilium. Thus, the loss of mating reactivity effected by these enzymes results initially from an alteration in the surface structure of these organelles. Interpretation of this effect in terms of a specific protein substrate presents certain difficulties, however. The proteolytic enzymes could act directly on the reactive groups (*i.e.*, the "mating substance"), in which case these reactive groups would be proteinaceous. On the other hand, the enzymes might destroy mating reactivity indirectly by breaking down protein in the superficial layers of the cilium. Such action might alter the relations (*i.e.*, spacing) of essential non-protein surface groups or even release the essential material into solution. This latter possibility was tested as outlined in the following section.

Failure of trypsin digests of reactive paramecia to act on animals of opposite type. Reactive *P. calkinsi* type I in four liters of culture were treated with formalin. The resulting reactive dead animals were washed in saline and pH 8 phosphate buffer. The final yield of 0.7 ml. of packed dead animals was taken up in one ml. of the buffer. Five-tenths ml. of this sample was mixed with an equal volume of 0.5 per cent trypsin in the same buffer. The remaining 0.5 ml. of suspension (control) was treated with buffer alone. The two suspensions were incubated at 32° C. for 5 hours. At the termination of this digestion period the enzyme-treated animals were reduced to fragile ghosts which gave no mating reactions. Animals of the control sample (treated with buffer alone) appeared to be in a good state of preservation and gave good mating reactions. The unreactive ghosts were now centrifuged from the trypsin-treated suspension and 0.25 ml. of one per cent trypsin inhibitor (ovomucoid prepared as described by Lineweaver and Murray, 1947) was added to prevent action of the enzyme on test animals. This trypsin inhibitor-treated, trypsin digest of formalin-killed *P. calkinsi* type I was tested for its ability selectively to destroy mating reactivity of type II animals. Eight drop samples of the digest were mixed with three drop samples from suspensions of diluted, freshly prepared, picric acid-killed reactive type I and II animals. One hour later these dead animals were washed in saline and tested for mating reactivity. The digest-treated type I and II animals both gave mating reactions of intensity equal to that of buffer-treated controls. Evidently, then, the trypsin digest of reactive type I animals has no mating reaction inhibiting properties.

Discussion

None of the several non-proteolytic enzymes employed had any detectable effect upon the mating reactivity of the paramecia. Assuming that these experiments were critical, this result indicates that the natural substrates of these enzymes are not essential constituents of the mating type substances of *P. calkinsi.* Elimination of these classes of substances as possibilities is not without interest since certain of them are known to be present in intercellular cement (hyaluronic acid) or cell membranes and cell surfaces (bacterial mucopolysaccharides hydrolyzed by lysozyme, Meyer and Hahnel, 1946; ribonucleic acid in Arbacia egg surface, Lansing and Rosenthal, 1949; lipoprotein attacked by lecithinase in erythrocyte and sea urchin egg membranes, Runnström, Tiselius and Lindvall, 1945).

As suggested elsewhere in this article the inactivating action of trypsin and chymotrypsin can be interpreted either as a direct effect upon the essential groups of the mating substance or an indirect action upon neighboring protein. Since no mating substance activity could be demonstrated either from the animals or the supernatant fluid following treatment with these enzymes, it appears that some essential molecular structure was destroyed. This suggests that the essential groups are protein or that they are intimately associated with protein. Insofar as the presence of immunizing antigens indicates protein, the immunological studies of Metz and Fusco (1948) support this view. It is of interest in this connection that the latter study indicates that the mating substance is associated with antigenic material but that the essential groups are not themselves antigenic.

Unfortunately, no more positive statement regarding the chemical nature of the mating substance seems warranted on the basis of the information presented here.

If mating substance activity is dependent upon protein integrity, as seems to be the case, then the protein material must be of rather unusual character. Its activity is not destroyed by fairly harsh treatment with certain strong protein-denaturing agents such as formalin, picric acid and mercuric chloride. Furthermore, the rate of inactivation of mating substance by trypsin and chymotrypsin may depend upon previous treatment (*i.e.*, formalin vs. picric acid). If true, this would also be an unusual situation since any substance or group of substances with as complex and highly specific action as these would be expected to show some differences in activity to correspond with differences in susceptibility to enzymes. Unfortunately no satisfactory method has been devised to assay mating substance activity. Therefore serious quantitative studies of the reactivity of formalin- as opposed to picric acid-killed animals, both before and after enzyme treatment, must await development of an appropriate technique. In view of these considerations, the possibility that the mating type substances of *P. calkinsi* are proteins is accepted as a working hypothesis with the customary reservations.

Aside from the question of the chemical nature of the mating type substances, the mating substance inactivating action of proteolytic enzymes is of interest in connection with the inhibiting action of certain paramecium extracts. Metz and Butterfield (1950) have shown that homogenates of *P. calkinsi* contain a heat-labile, non-type specific mating substance inhibiting agent. It was suggested that this agent might be an enzyme. Considering the present findings it seems possible that the agent in question is a proteolytic enzyme. Such an enzyme need not bear any physiological relation to the mating substances. Indeed, it may well be a proteolytic enzyme which is released into the food vacuoles in the living animals and which under normal conditions participates in the digestion of the contents of these vacuoles (see Mast, 1947). Upon homogenizing the paramecia, such an enzyme could be released into the extraction fluid and under the conditions of the experiments would be expected to inactivate the mating substances of the test animals.

Summary

1. In an attempt to characterize the mating type substances of *Paramecium calkinsi*, several enzymes were tested for ability to destroy the reactivity of formalin- or picric acid-killed animals.

2. The following non-proteolytic enzymes had no detectable effect upon the mating reactivity of *P. calkinsi*: Lecithinase (bee venom), hyaluronidase, lysozyme, ptyalin, ribonuclease. Insofar as these experiments were critical, it is concluded that the natural substrates for these enzymes are not essential constituents of the mating type substance of *P. calkinsi*.

3. Crystalline preparations of the proteolytic enzymes trypsin and chymotrypsin destroyed the mating reactivity of both type I and type II animals. Mating substance activity was not found in the digest of enzyme-treated paramecium.

4. On the basis of these findings it is concluded that mating reactivity is dependent upon protein integrity. Possibilities regarding the nature of this dependence are discussed.

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NEUROSECRETION. X. A RELATIONSHIP BETWEEN THE PARAPHYSIS AND THE PARAVENTRICULAR NUCLEUS IN THE GARTER SNAKE (THAMNOPHIS SP.) [1]

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In an earlier paper in this journal (Scharrer and Scharrer, 1944) it was shown that in vertebrates the stainable material produced by the neurosecretory cells of the hypothalamus passes along the axons of these cells toward the hypophysis. Similar material elaborated by the neurosecretory cells of the pars intercerebralis in insects also "migrates" along nerve fibers toward an organ complex, namely the corpora cardiaca and allata, which corresponds in many ways to the hypophysis of the vertebrates. The concept of two analogous neuroendocrine systems in invertebrates and vertebrates, which was established on the basis of these observations, has proved fruitful. As to the intercerebralis-cardiacum-allatum system of the insects, Thomsen (1948) studied a possible relationship between the neurosecretory activity of the pars intercerebralis and the "gonadotropic" principle of the corpus allatum. The study showed a positive relationship in that extirpation of the pars intercerebralis in the fly, *Calliphora erythrocephala,* prevented the production or release of the gonad-stimulating principle of the corpus allatum and resulted in the inability of the ovary to produce mature eggs. Also, the concept of an analogous hypothalamo-hypophyseal system in vertebrates has been extended further by Bargmann (1949), Bargmann and Hild (1949), Hild (1950), Ortmann (1950), Bargmann, Hild, Ortmann and Schiebler (1950) and Smith (1951). There is now convincing evidence, recently reviewed by Bargmann and Scharrer (1951), that the stainable material produced by the secreting nerve cells of the hypothalamus and passing along their axons to the neurohypophysis is related to the hormones which can be extracted from the pars nervosa of the pituitary gland.

If this conclusion is correct, neither the corpus cardiacum of the insects nor the neurohypophysis of the vertebrates are endocrine organs in a strict sense, but serve principally as sites of storage of the products of neurosecretory cells in the brain.

Questions arise as to whether (a) the neurohypophysis and the corpus cardiacum are the only organs into which the secreting cells of the hypothalamus and the pars intercerebralis respectively discharge their products, and (b) neurosecretory centers other than those in the hypothalamus of the vertebrates and the pars intercerebralis of the insects are also connected with reservoirs which receive the products of neurosecretory activity by way of axons and store them for release according to need. Both problems are at present under investigation. Observations reported in this paper concern a relationship between the neurosecretory cells of the paraventricular nucleus and an organ other than the pars nervosa of the pituitary, namely the paraphysis, in the garter snake.

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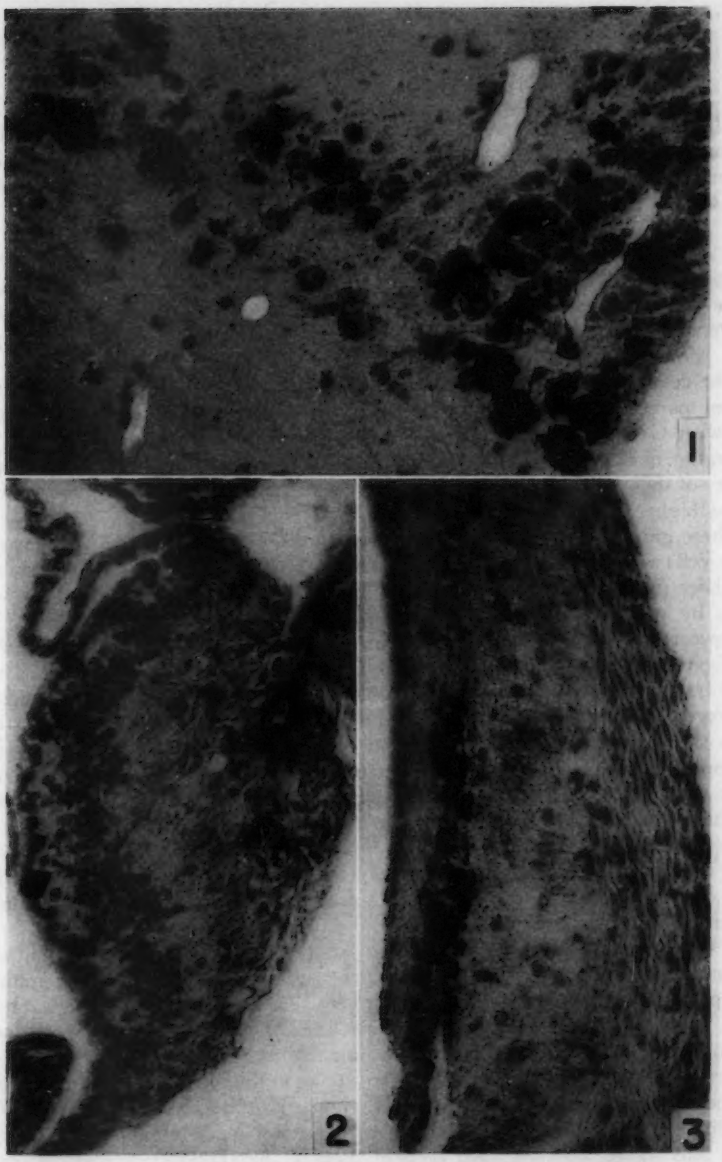

FIGURES 1 to 7: Thamnophis sp., 5 micra sections; Gomori's chrome alum hematoxylin phloxine; photomicrographs.

FIGURE 1. Nucleus supraopticus. Secretory granules and droplets are stained deep blue. × 440.

FIGURE 2. Cross section of stalk of pituitary with stainable material from neurosecretory cells in the hypothalamus. × 400.

FIGURE 3. Longitudinal section through stalk of pituitary with stainable material from neurosecretory cells in the hypothalamus. × 400.

Materials and Methods

The brains of garter snakes (Thamnophis sp.) were fixed with Zenker-formol by immersion or perfusion through the heart. They were embedded in paraffin; sections of 5 micra thickness were stained with chrome alum hematoxylin phloxine as described by Gomori (1941).

Observations

In snakes, as in higher vertebrates, there are two nuclei in the hypothalamus whose cells show secretory activity: the nucleus supraopticus and the nucleus paraventricularis (Scharrer, 1933). The location of these nuclei in the garter snake is illustrated in Figure 8.

The cells of the supraoptic and paraventricular nuclei are distinct in that they contain many fine granules and droplets of varying size. This material stains dark blue with Gomori's chrome alum hematoxylin phloxine method (Fig. 1). The elaboration of this stainable material in granular form is characteristic of the secretory activity of these cells, not only in the snakes but in the homologous nuclei of lower as well as higher vertebrates.

These granules, as in the case of other animals, are contained in the processes of the cells which can, therefore, be traced with great accuracy. It was shown in an earlier paper of this series (Palay, 1945) that in fishes the axons provide a means by which the granules are removed from the neurosecretory cells. This "neurosecretory pathway" in snakes, as in other vertebrates, leads to the pars nervosa of the pituitary gland (Bargmann, Hild, Ortmann and Schiebler, 1950). That an actual transport takes place by a proximo-distal current of axoplasm was suggested by Drager (1950). He replaced the pituitary gland by fibrin foam in the tropical indigo snake (*Spilotes corais*) and found two to three weeks after the operation in the non-living implant granules of a stainable material similar to that elaborated by the neurosecretory cells. Histological observations in the garter snake indicate that the mechanism is the same as in the tropical indigo snake. The pituitary stalk of the garter snake contains large amounts of the stainable material (Figs. 2, 3) which is also found in the neurosecretory cells of the supraoptic and paraventricular nuclei. This material eventually accumulates in the pars nervosa of the hypophysis.

A small number of fibers which arise from cells in the anterior portion of the nucleus paraventricularis and run anteriorly and dorsally toward the commissura pallii posterior (Kappers, Huber and Crosby, 1936, p. 1347) and the paraphysis (Figs. 4, 5), constitute another, hitherto unknown, connection along which neurosecretory granules may be traced. The stainable material accumulates in the commissura pallii posterior in much the same way as in the pituitary stalk (Figs. 6, 7); the amounts of the material vary from individual to individual (Figs. 5, 6). Fibers from the commissura pallii posterior carrying small blue-staining granules can be traced into the paraphysis. No accumulation of the stainable substance seems to take place in the paraphysis; here the granules appear to fade out.

Although the quantities of stainable material involved in the pathway from the paraventricular nucleus to the paraphysis are small by comparison with those seen in the hypothalamo-hypophyseal system, a similar principle seems to obtain in both cases. This similarity of the dorsal and ventral outlets for the secretory products of

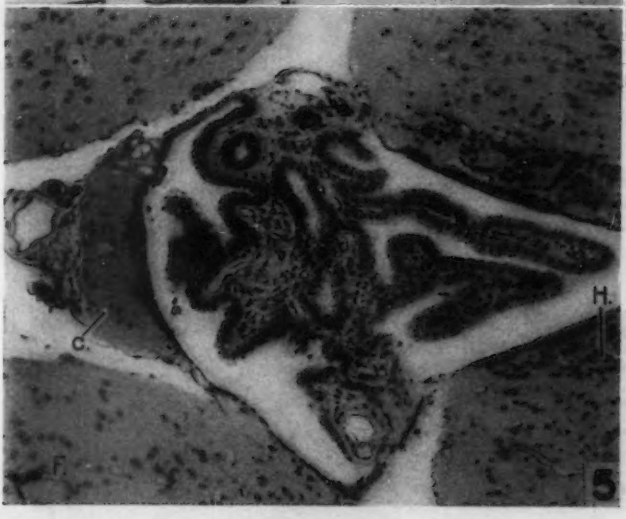

FIGURE 4. Sagittal section through diencephalon. × 40. C., Commissura pallii posterior; E., Epiphysis; M., Midbrain; O., Optic chiasm; P., Paraphysis; PN., Paraventricular nucleus; S., Stalk of pituitary.

FIGURE 5. Basiparallel section through the epithalamus. Note the small amount of stainable material in the commissura pallii posterior as compared with the large amount in the animal shown in Figures 6 and 7. × 180. C., Commissura pallii posterior; F., Forebrain; H., Habenular ganglion.

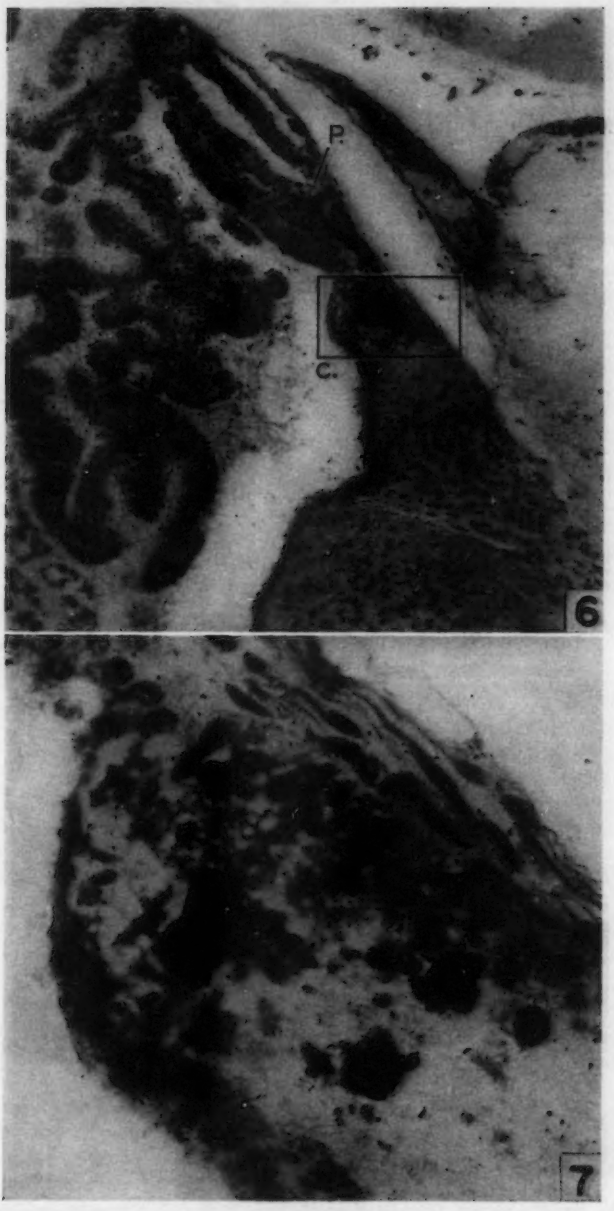

FIGURE 6. Sagittal section through the epithalamus. × 180. C., Commissura pallii posterior; P., Paraphysis.

FIGURE 7. Accumulation of stainable material in the Commissura pallii posterior in the same animal shown in Figures 4 and 6. × 1000.

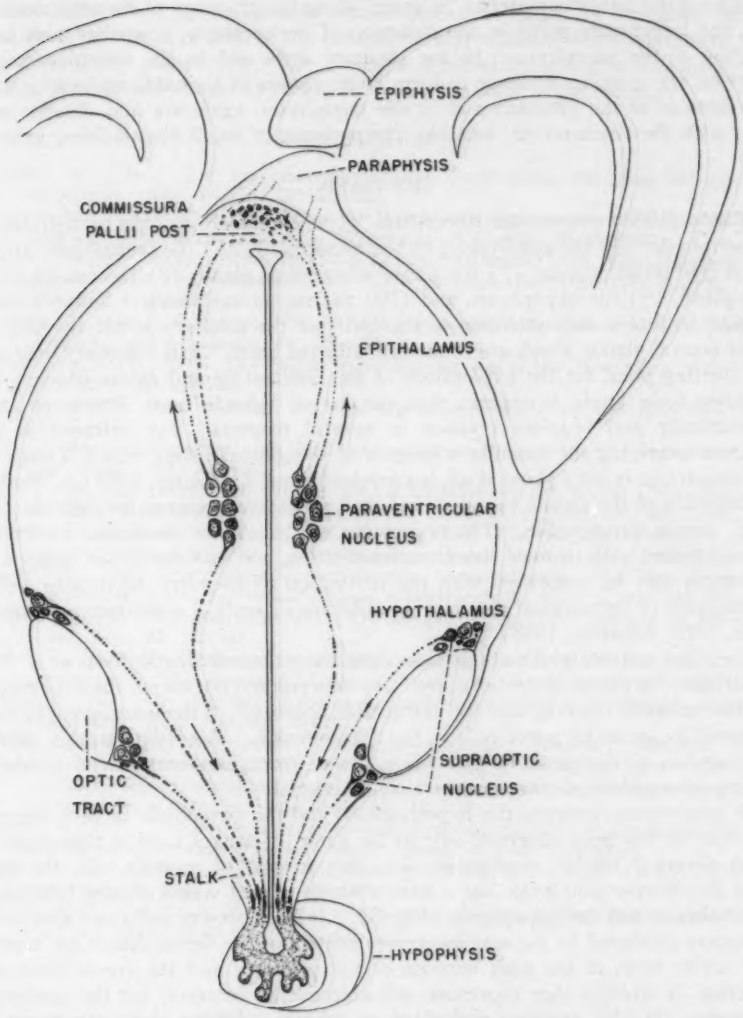

FIGURE 8. Diagram of cross section through the diencephalon of Thamnophis sp. The irregularly spaced dots represent granules of stainable material which mark the axons of neurosecretory cells of the paraventricular and supraoptic nuclei. These granules pass from the neurosecretory cells by way of the axons to (a) the neurohypophysis and (b) the commissura pallii posterior and the paraphysis. The arrows indicate the direction of flow.

the hypothalamus is diagrammatically illustrated in Figure 8. In both cases fine granules of the stainable material "migrate" along the processes of the neurosecretory cells, the larger part going in the direction of the pituitary, a smaller part in the direction of the paraphysis. In the pituitary stalk and in the commissura pallii posterior, the granules coalesce to form large masses of stainable material. In the pars nervosa of the pituitary and in the paraphysis, again we find delicate nerve fibers with the characteristic beadlike arrangement of small blue-staining granules.

Discussion

Tilney (1938) enumerates 10 cerebral "glands," namely (1) the paraphysis, (2) the periphysis, (3) the epiphysis, (4) the mesophysis, (5) the metaphysis, (6) the lateral chorioideal glands, (7) the mesial chorioideal gland, (8) the caudal chorioideal gland, (9) the hypophysis, and (10) the saccus vasculosus. Tilney's outline is useful in that it calls attention to the fact that the pituitary is not the only, but one of several glands which are connected with the brain. His summary may serve as a starting point for the exploration of this interesting and rather obscure field, but after some study it appears that the list of "glands" was drawn up rather schematically and requires revision in several respects. For instance, a large structure overlying the medulla oblongata in the ganoid fishes, which Tilney calls the metaphysis, is not a gland at all, but myeloid tissue (Scharrer, 1944). Similarly, the inclusion of the saccus vasculosus of the teleost fishes among the "glands of the brain" seems questionable. The occurrence of the saccus vasculosus in deep sea fishes, together with its finer structure, innervation, and vascularization suggest that this organ may be concerned with the perception of pressure differences and the maintenance of intracranial pressure equilibrium in vertical movements (Dammermann, 1910; Scharrer, 1948).

Even the criteria which are used in classifying the neurohypophysis as a "gland of the brain" are now under scrutiny. As was pointed out above there is evidence that the stainable material and the extractable hormones of the neurohypophysis are produced by secreting nerve cells in the diencephalon. According to this view the pars nervosa of the pituitary gland is a site of storage rather than of production of hormones and of microscopically visible material.

A relationship between the hypothalamus and the paraphysis is here suggested which so far has been observed only in the genus of snakes used in this study, but which deserves further exploration in other groups of animals. In the garter snake this relationship looks like a mirror image of that which obtains between the hypothalamus and the hypophysis (Fig. 8). It is at present not clear whether the substances produced by the neurosecretory centers of the diencephalon are stored in their active form in the pars nervosa of the pituitary and the commissura pallii posterior, or whether they represent only antecedents necessary for the synthesis of hormones. It also remains undecided at present whether these structures, the pars nervosa and the commissura pallii posterior, together with the paraphysis, serve no other purpose than to store and release the substances which they receive from the neurosecretory cells or whether they themselves produce additional material.

Summary

In snakes, as in other vertebrates, the neurosecretory cells of the supraoptic and paraventricular nuclei discharge their products by way of their axons into the pars

nervosa of the pituitary gland. In the garter snake (Thamnophis sp.) another, hitherto undescribed neurosecretory pathway exists: secretory granules can be traced along processes of cells of the paraventricular nuclei to the commissura pallii posterior and the paraphysis. The granules accumulate in the commissura pallii posterior in a manner similar to that observed in the stalk of the pituitary.

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CHARACTERISTICS OF THE RETINAL ELECTRIC RESPONSE OF THE OCELLI OF LIMULUS

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The median ocelli of *Limulus polyphemus*, located one on each side of a spine on the dorsal and anterior surface of the carapace, are well suited for investigations into the electro-physiological properties of photoreceptors. These small clusters of sense cells, about 50–80 in number, can be dissected free of the cornea and surrounding connective tissue quite readily. The free ocellus, about 0.5 mm. in diameter, can then be drawn into a tapering capillary tube. Such a preparation, providing it is not subjected to unreasonable abuse, will give reproducible electrical responses upon illumination for periods up to six hours.

The data presented below were obtained from ocelli treated in the manner just described. A capillary containing the ocellus was sealed into a small hole in the partition which separated two compartments of a plastic electrode chamber. The light-receiving end of the ocellus was in contact with sea water of the forward compartment and the terminal portion of the nerve was in contact with sea water in the rear compartment. Each compartment contained a silver-silver chloride electrode in contact with the sea water.

The light source used in this experiment was a 500 watt tungsten filament projection bulb. At the focal point the maximal intensity was 27,000 foot candles and this was considered unit intensity. To cool the light beam it was passed through 8 centimeters of water and a heat-absorbing filter. The intensity of the light beam was reduced by Wratten neutral tint filters. The duration of the light flash was controlled by a photographic shutter. A portion of the light beam was diverted to a photocell, the output of which was recorded as the stimulus signal. The nominal exposures of the shutter were calibrated by photographing a cathode ray beam deflected by the output of the photocell.

The potentials appearing across the eye on illumination were fed into high gain, condenser-coupled amplifiers and recorded on an ink writer. The recording paper speed was 25 centimeters per second; the paper speed was calibrated with a 60 cps. signal. The ink writer responded faithfully to calibrating signals up to 60 cycles per second. The deflections of the writing pens were calibrated with square wave voltage pulses and a 60 cycle per second signal after each exposure. The time constant of the amplifiers was 0.5 second. It is felt that the distortion of the wave form of the retinal action potentials caused by the 0.5 second time constant does not seriously affect the results obtained nor the interpretation of the results.

After dark-adaptation was complete, a flash of light of the lowest intensity to be used was admitted and the response recorded. For relatively low intensities and/or short durations (less than 0.1 second) of illumination, 10 minutes between flashes

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were found to be sufficient for recovery. For higher intensities and/or for longer durations (more than 0.1 second) of illumination, it was necessary to allow 15 to 20 minutes between flashes.

Results

The wave form of the response

The time course of the retinal action potential obtained upon brief illumination of an excised median ocellus is indicated in Figure 1. The smooth contour of the response is similar to the second response curve obtained from the lateral eye of

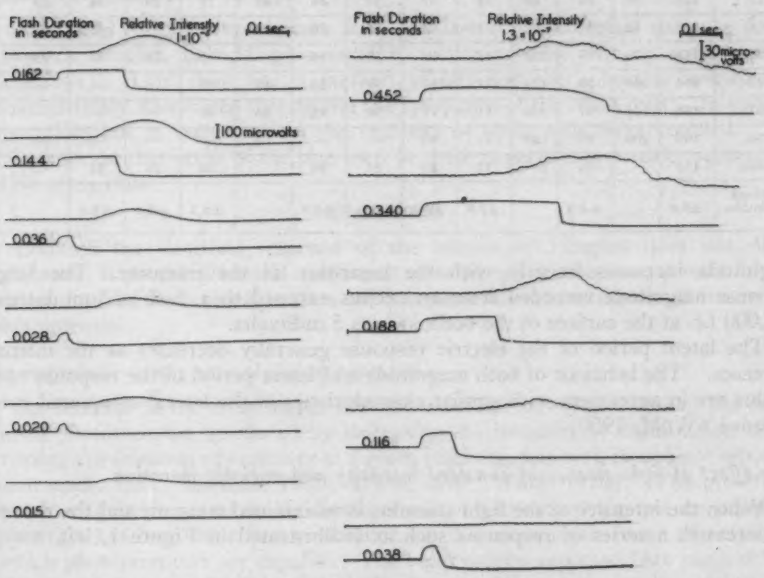

FIGURE 1. Right: Photographs of retinal action potentials elicited from an isolated ocellus illuminated with low intensity for varying durations. The duration in seconds is given on the lower tracing of each pair of records, which is the record of the output of the photocell. The ocellus was maximally dark adapted before each flash.

Left: Photographs of retinal action potentials from an ocellus illuminated with a moderate intensity for varying durations. Records marked as above. Refer to text for further detail.

Limulus (Graham, 1932; Wulff, 1950). Light flashes of very low intensity (0.027 f.c. or less) often elicit responses of irregular contour (Fig. 1, right). As the flash duration is increased, the response spreads out in time and the irregularity increases. If the intensity of illumination is increased, the irregularity of the response disappears.

The effect of light flashes of constant duration and variable intensity

A light stimulus of the lowest useful intensity generally elicits a response with crest magnitudes of about 10 microvolts. As the intensity of illumination is increased, the response magnitude increases also, the relation being such that the

TABLE I

Latent periods and crest times obtained during a typical experiment with an excised median ocellus of Limulus. Relative intensities are at the top of each column. All times are in milliseconds. The means and their standard deviation are given underneath particular columns

Flash Duration msec.	1×10^{-6}		1×10^{-5}		1×10^{-4}		1×10^{-3}		1×10^{-2}		1×10^{-1}		1×10^{0}	
	Latent Period	Crest Time	Latent Period	Crest Time	Latent Period	Crest Time	Latent Period	Crest Time	Latent Period	Crest Time	Latent Period	Crest Time	Latent Period	Crest Time
17	128	96	80	80	72	92	52	84	40	72	28	36	23	32
21	120	60	85	92	73	83	56	76	40	64	29	35	23	32
32	118	94	89	112	68	84	56	84	—	—	28	32	23	32
42	104	100	88	124	72	84	56	88	40	68	—	—	—	—
101	108	125	91	120	71	77	56	92	40	76	—	—	—	—
256	100	200	84	129	74	82	—	—	—	—	—	—	—	—
Mean	113		86		72	84	55	85		70	28	34		
Standard Deviation	±9.8		±3.6		±1.9*	±4.4	±1.6	±4.8		±6.3	±0.6	±1.6		

magnitude increases linearly with the logarithm of the intensity. The largest response magnitude recorded from an ocellus exposed to a flash of unit intensity (27,000 f.c. at the surface of the ocellus) was 5 millivolts.

The latent period of the electric response generally decreases as the intensity increases. The behavior of both magnitude and latent period of the response of the ocellus are in agreement with similar characteristics of the lateral compound eye of Limulus (Wulff, 1950).

The effect of light flashes of constant intensity and variable duration

When the intensity of the light stimulus is maintained constant and the duration is increased, a series of responses such as is illustrated in Figure 1, left, may be

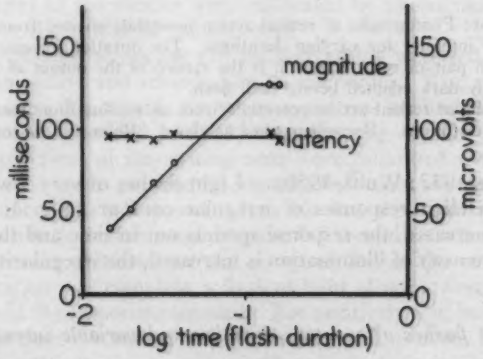

FIGURE 2. Magnitude of the crests and latencies of the responses of Figure 1, left, plotted as a function of the common logarithm of the flash duration. Refer to text for further detail.

obtained. The latent period of the responses is constant for all flash durations at the same intensity level (Fig. 1 and Table I). It is only when the intensity level is changed that the latent period changes.

The magnitude of the responses illustrated in Figure 1, left, gradually increases to a maximal value which is reached when the flash duration is about 0.1 second (see Fig. 2). Light flashes of duration longer than 0.1 second do not produce any further increase in magnitude. The influence of flash duration on response magnitude has been determined for the grasshopper eye by Hartline (1928).

Histological examination of ocelli

Ocelli used in these experiments were prepared for histological study by fixing in Bouin's solution, imbedding in paraffin, sectioning and staining with thionine ($C_{12}H_9N_3S$). This stain has a marked affinity for the spherical rose-colored inclusions richly distributed throughout the cytoplasm of the sense cells. In suitable preparations cut in cross section, the numbers of sense cells were counted. The results indicate that ocelli of the size used in these experiments contain between 50 and 80 sense cells.

Discussion

Although the electrical response of the ocellus of Limulus does not differ significantly from the electrical responses of other photoreceptors, certain of the observations lead to a critical evaluation of some of the characteristics of the retinal action potential.

The magnitude of the retinal action potential

The increase in the crest magnitude of the retinal action potential of the dark-adapted photoreceptor, produced by increasing the intensity of illumination or by increasing the duration of exposure at a given intensity, has been frequently reported (Kohlrausch, 1931; Hartline, 1928; Granit, 1947; Wulff, 1950). The growth of response magnitude seems to be characteristic of photoreceptors, yet no adequate explanation has been advanced to account for the tremendous range of responses of which photoreceptors are capable. The observations reported here suggest that the increase in magnitude of the electrical response of the median ocellus of Limulus may be primarily the result of graded responses on the part of the sense cells, with the added possibility that sense cell recruitment may occur at low intensities of illumination.

That the sense cells of the median ocellus of Limulus can produce responses of graded magnitude is indicated by the following observations. The maximal response recorded from an isolated ocellus was of the order of 5 millivolts. Minimal response magnitudes that could be measured with reliability were of the order of 0.01 millivolt. Should 0.01 mv. represent the maximal (all or none) response of a single sense cell then, assuming the best possible conditions for summation, the number of sense cells required to produce a response of 5 mv. would be 500. Actual counts of sense cells in ocelli used range between 50 and 80, a number entirely inadequate to account for the observed results unless each sense cell is capable of producing a response whose magnitude varies with the intensity, the duration of illumination or both.

The recruitment of sense cells by light flashes of long duration incident on an excised median ocellus of Limulus is illustrated in Figure 1, right. The extreme

temporal dispersion of discrete electrical waves indicated in Figure 1 is observable only at low levels of illumination. Increasing the intensity of illumination generally produces a smooth response regardless of the exposure (Fig. 1, left). However, the crests of the smooth responses shift in time as the light flashes increase in duration so that the crest time, measured from onset of response to the crest, increases (Table I). The shift in crest time is apparent only at the lower intensities of illumination (Table I) and it is assumed that recruitment of sense cells occurs at those intensities where the crest time increases with increasing flash duration.

The retinal action potential and the optic nerve discharge

It has been suggested (Hartline, 1935; Wulff, 1943; Granit, 1947) that the retinal action potential generates local currents which initiate impulses in the optic nerve. In the light of such an hypothesis a large retinal action potential would set up more intense local currents than a smaller potential and hence the resultant nerve impulse discharge might begin sooner in time and be more intense, *i.e.,* the frequency of the impulses might be greater. Hartline (1934) has measured the characteristics of the nerve impulses discharged into a single optic nerve fiber in response to light flashes of different intensities and durations. The results of Hartline's observations that pertain to this discussion are: 1) The frequency of the first three impulses of the train of impulses increases with increasing flash duration, reaching a value that is roughly constant at flash durations of 0.1 second and higher; 2) The latency of the optic nerve discharge decreases as the flash duration increases, reaching values roughly constant for flash durations of 0.1 second and higher. Assuming for the moment that the characteristics of the ocellus of Limulus are applicable to the lateral eye, then the results of Hartline's experiment are in accord with the hypothesis that the retinal action potential initiates activity in the optic nerve because: 1) the direction of the changes in latency and frequency of the optic nerve discharge produced by light flashes of increasing duration is in harmony with the increase in magnitude of the retinal action potential; and 2) the salient characteristics of both the retinal action potential and the optic nerve discharge are constant for light flashes of durations of 0.1 second and higher. Very recently it was demonstrated that the response characteristics of the lateral eye of Limulus are in accord with the characteristics of the ocellus.

The constant latent period

The results reported above indicate that (within the limits of exposures available) the latent period of the retinal action potential is an inverse function of the intensity of illumination and is quite independent of the duration of illumination. Since the magnitude of the response increases to a maximal value at any given intensity for flash duration of 0.1 second or greater and the latent periods of the responses are quite constant (Table I), the possibility is suggested that the onset of the response and the magnitude of response may be controlled by separate events in the sense cells. The validity of this suggestion must be tested by further investigation.

Another interpretation of these results may be based on the existence of a sensitization period, first described by Hecht (1918, 1919). According to Hecht, the sensitization period is that period during which the photoreceptor must be illuminated

in order to produce a response. The duration of such a sensitization period in the Limulus ocellus might well be shorter than the shortest flash duration available, and it is conceivable that this sensitization period may determine the latency, *i.e.*, at any given intensity, a response of fixed latency either will occur or else there will be no response at all. Data obtained from the ocellus of Limulus at low levels of illumination (Fig. 1) indicate that the response magnitude for short exposures tends to approach the abscissa (zero microvolts) very gradually; there is no suggestion of a sudden drop that might be expected if a sensitization period actually exists.

Summary

1. The electrical responses to illumination were obtained from isolated median ocelli of *Limulus polyphemus* mounted in a capillary tube filled with sea water. These preparations, maintained in a state of dark adaptation, were periodically illuminated at different intensities for different durations.

2. For the most part, the reported data are in accord with similar data obtained from the lateral compound eye of Limulus and the compound eyes of grasshoppers. It was observed that the latent period of the responses elicited by light flashes of different durations at any one intensity is constant; the only factor seemingly affecting the latent period is the intensity.

3. The increase in electrical response magnitude produced either by increasing the intensity and/or the duration of illumination, which is typical of many photoreceptors, is accounted for on the basis of graded responses on the part of sense cells. The possibility exists that recruitment of sense cells is a contributing factor at low intensities of illumination.

4. Comparison of data obtained from the median ocellus of Limulus with data obtained from single optic nerve fibers of the lateral eye of Limulus indicates a parallelism which is in accord with the hypothesis that the retinal action potential sets up local action currents which initiate impulses in the optic nerve.

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